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(54) Title: USE OF CO-LOCALIZED ISLETS AND SERTOLI CELLS IN XENOGRAFT CELLULAR TRANSPLANTS			
(57) Abstract <p>The present invention describes a method of treating a disease that results from a deficiency of a biological factor which comprises administering to a mammal Sertoli cells and cells that produce the biological factor. In particular, the present invention describes a method of treating diabetes mellitus by transplanting pancreatic islet of Langerhans cells in conjunction with Sertoli cells to create an immunologically privileged site. A method of creating an immunologically privileged site and providing cell stimulatory factors in a mammal for transplants is further described by the present invention. A method of co-localizing islet cells with Sertoli cells and the use of the co-localized product in treating diabetes mellitus is further provided. The present invention further describes a method of creating systemic tolerance to foreign antigens. A method of enhancing the viability, maturation, proliferation of functional capacity of cells in tissue culture is further provided. A pharmaceutical composition comprising Sertoli cells and cells that produce a biological factor is also provided.</p>			

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1 USE OF CO-LOCALIZED ISLETS AND
 SERTOLI CELLS IN XENOGRAFT CELLULAR TRANSPLANTS

 This invention was made with United States
government support under grant DK42421 awarded by the
5 National Institutes of Health. The United States
Government may have certain rights in the invention.

CROSS-REFERENCE OF RELATED APPLICATIONS

10 This application is a continuation-in-part
of U.S. Serial No. 08/485,340 filed on June 7, 1995
which is a continuation-in-part of U.S. Serial No.
08/421,641 filed on April 13, 1995 which is a
continuation-in-part of U.S. Serial No. 08/211,695
15 filed on April 13, 1994.

FIELD OF THE INVENTION

 Transplants of healthy organs or cells into
a patient suffering from a disease are often rejected
by the body due to an immune response initiated in
20 response to the foreign tissue or cells. The present
invention provides a method of cellular
transplantation in which an immunologically privileged
site is created and cell stimulatory factors are
produced, thus alleviating the rejection associated
25 with conventional transplantation therapy.
Specifically, the present invention describes a method
of treating a disease that results from a deficiency
of a biological factor which comprises administering
to a mammal Sertoli cells and cells that produce the
30 biological factor. In particular, the present
invention describes a method of treating diabetes

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1 mellitus by transplanting pancreatic islet of
Langerhans cells in conjunction with Sertoli cells to
create an immunologically privileged site and to
provide pancreatic islet cell stimulatory factors. A
5 method of creating an immunologically privileged site
and providing cell stimulatory factors in a mammal for
transplants is further described by the present
invention. A method of creating systemic tolerance to
transplants is further provided by the present
10 invention. The present invention further describes a
method of enhancing the maturation, proliferation and
functional capacity of cells in tissue culture by co-
culturing these cells with Sertoli cells. A method of
enhancing the recovery rate and viability of frozen
15 cells, and in particular factor producing cells, in
tissue culture by co-culturing these cells with
Sertoli cells is also described herein. Another
aspect of the present invention is directed to a
method of co-localizing Sertoli cells with cells that
20 produce a biological factor for treating diseases
caused by a deficiency thereof, e.g., encapsulating
islet cells which produce insulin with Sertoli cells.
The use of the co-localized, e.g., encapsulated
Sertoli cells and islet cells for treating diabetes
25 mellitus is further described by the present
invention. A pharmaceutical composition comprising
Sertoli cells and cells that produce a biological
factor is also provided.

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1 BACKGROUND OF THE INVENTION

Certain chronic diseases destroy the functional cells in affected organs. Mammals with such diseases are often unable to produce proteins or hormones necessary to maintain homeostasis and usually require numerous exogenous substances to survive. Transplanting healthy organs or cells into a mammal suffering from such a disease may be necessary to save the mammal's life. This type of therapy is generally regarded as a last alternative to curing an otherwise fatal condition. Such transplants, however, are often rejected by the body due to an immune response initiated in response to the foreign tissue or cells. Presently, the only recourse to combat this immune response is to administer chronic nonspecific immunosuppression agents. Unfortunately, this only trades the complications of one chronic disease with other complications caused by the immunosuppression agent.

One disease which scientists have attempted to treat with organ and/or cellular transplants but have had very limited success is diabetes mellitus. Diabetes mellitus is a prevalent degenerative disease in mammals. It is characterized by a relative or complete lack of insulin secretion by the beta cells within the islets of Langerhans of the pancreas or by defective insulin receptors.

This insulin deficiency prevents normal regulation of blood glucose levels and often leads to hyperglycemia and ketoacidosis. When administered to a mammal, insulin promotes glucose utilization,

1 protein synthesis, formation and storage of neutral
lipids and the growth of certain cell types.

In the United States alone there are
approximately 13 million diabetics. Of these, 2.6
5 million are insulin dependent diabetics. Drug &
Market Dev., 4:210 (1994). Health care analysts
estimate that diabetes costs \$92 billion a year
resulting from medical costs and lost productivity.

The various forms of diabetes have been
10 organized into a series of categories developed by the
National Diabetes Data Group of the National
Institutes of Health. Type I diabetes in this
classification scheme includes patients dependent upon
insulin to prevent ketosis. This group of diabetics
15 was previously called juvenile-onset diabetes, brittle
diabetes or ketosis-prone diabetes. Type I diabetes
is caused by an autoimmune reaction that causes
complete destruction of beta cells.

Type II diabetes is classified as adult-
20 onset diabetics. The diabetic patient may or may not
be insulin dependent. Type II diabetes can be caused
by a number of factors. For most mammals with Type II
diabetes, the beta islet cells are defective in the
secretion of insulin.

25 There are many therapies currently used to
treat diabetes, however, each has its limitations.
The major problem confronting most patients with
diabetes mellitus is that currently available
therapies fail to prevent the complications of the
30 disease process. The most common method of treating
Type I diabetes in mammals is providing an endogenous

1 source of insulin such as porcine, bovine or human
insulin. Insulin injection therapy prevents severe
hyperglycemia and ketoacidosis, but does not
completely normalize blood glucose levels. This
5 treatment further fails to prevent the complications
of the disease process, including premature vascular
deterioration. Premature vascular deterioration is
the leading cause of morbidity among diabetic
patients. Furthermore, complications resulting from
10 long-term diabetes include renal failure, retinal
deterioration, angina pectoris, arteriosclerosis,
myocardial infarction and peripheral neuropathy.

A second method of treating diabetes is by
transplanting the pancreas in conjunction with the
15 administration of chronic nonspecific
immunosuppression agents. This treatment is usually
given to an individual who has advanced diabetes, such
as an individual with kidney failure. Whole pancreas
transplantation can be successfully done with a 75%
20 one year survival rate, but surgical transplantation
of the pancreas is very difficult. Furthermore, since
the entire organ must be donated, the only practicable
source is a deceased donor. In addition, when
cyclosporine, the most common immunosuppressive drug
25 used for organ transplants, is administered in a
dosage necessary to suppress the immune response, the
drug inhibits pancreatic cell function. Furthermore,
the steroids that are often administered with an organ
transplant often cause the patient to become diabetic.

30 A third treatment involves transplanting
islet of Langerhans cells into the diabetic patient.

1 However, islet transplantation has been generally
unsuccessful due to the aggressive immune rejection of
islet grafts. (Gray, 1991, Immunology Letters 29:153;
5 Jung et al., 1990, Seminars in Surgical Oncology
6:122). In particular, successful transplantation of
isolated pancreatic islet cells has been very
difficult to achieve due to the chronic administration
of immunosuppressive drugs required to prevent organ
rejection of the cells following transplantation.
10 These dosages of immunosuppressive drugs can cause
increased susceptibility to infection, hypertension,
renal failure and tumor growth. Furthermore, unlike
most organ transplants, islet cells must grow their
own blood supply following implantation in the host in
15 order for the cells to survive. Conventional
transplantation techniques do not provide the
necessary factors to stimulate the production of new
blood vessels.

Thus, to successfully transplant cells in a
20 mammal, it is necessary that the cellular transplants
are not rejected by the recipient and have the
capacity to grow upon transplantation. As a
commercial reality, it is further necessary that a
sufficient quantity of cells are available for
25 transplantation. Traditionally, the number of
cellular transplants have been limited by the
inability to adequately collect and store a sufficient
number of cells for transplantation. Conventional
storage techniques, such as cryopreservation, often
30 damage a large quantity of the stored cells. Porcine
islet cells, for example, are extremely fragile and

1 easily dissociate into fragments or single cells upon
thawing.

The present invention alleviates many of the
problems associated with the current therapies for
5 chronic diseases that destroy the functional cells of
vital organs. Specifically, the present invention
provides a method of creating systemic tolerance to
subsequent transplants in the mammal. Furthermore,
the present invention solves the problems associated
10 with the conventional therapies for diabetes mellitus,
by providing a method of transplanting pancreatic
islets cells into a diabetic mammal, whereby the
cellular transplants produce insulin in the diabetic
mammal. The present inventor has previously
15 demonstrated extended functional survival of islet
cells allografts and xenografts in the testis.

(Selawry et al., 1989, Diabetes 38:220.) It has been
surprisingly discovered in accordance with the present
invention that an immunologically privileged site can
20 be created in a mammal by transplanting Sertoli cells
to a nontesticular site in a mammal. The newly
created immunologically privileged site allows the
transplantation and survival of cells that produce
biological factors useful in the treatment of
25 diseases, especially diabetes. In addition to
creating an immunologically privileged site, the
Sertoli cells produce cell stimulatory factors which
enhance the maturation, proliferation and functional
capacity of cells. Sertoli cells have further been
30 found to enhance the recovery rate and viability of

1 mammalian cells stored by techniques such as
cryopreservation.

SUMMARY OF THE INVENTION

5 The present invention relates to a method of
treating a disease that results from a deficiency of a
biological factor in a mammal which comprises
administering Sertoli cells and cells that produce the
biological factor. In a preferred embodiment, the
10 biological factor is a hormone.

In a more preferred embodiment, the disease
is diabetes mellitus, the factor producing cells are
pancreatic islet cells and the factor is insulin.

In yet another embodiment the cells that
15 produce the biological factors are cells that have
been genetically engineered, for example by
transformation with a nucleic acid that expresses the
biological factor.

The present invention further relates to a
20 method of treating diabetes mellitus in a mammal
comprising administering pancreatic islet cells and
Sertoli cells. In a preferred embodiment the Sertoli
cells and islet cells are administered by
transplantation. The Sertoli cells may be isolated
25 from a mammal or they may be derived from a Sertoli
cell line, in accordance with the present invention.

Another aspect of this invention is directed
to a method of creating an immunologically privileged
site and producing cell stimulatory factors in a
30 mammal.

1 A further aspect of the present invention is
directed to a method of creating systemic tolerance to
a subsequent transplant in a mammal by transplanting
Sertoli cells prior to said subsequent transplant.

5 Still a further aspect of the present
invention provides a method of enhancing the
maturation, proliferation and functional capacity of
cells in tissue culture by co-culturing these cells
with Sertoli cells.

10 A method of enhancing the recovery rate and
viability of frozen mammalian cells and in particular
factor producing cells, in tissue culture by co-
culturing these cells with Sertoli cells is further
provided by the invention described herein.

15 Another aspect of the present invention is
directed to a method of co-localizing, e.g.,
encapsulating the biological factor producing cells,
e.g., islet cells, with Sertoli cells and to the use
of the co-localized product for enhancing long-term
20 immunoprotection and nutritional survival of islets
and for the treatment of diabetes.

 Yet another embodiment of the present
invention provides a pharmaceutical composition
comprising Sertoli cells and cells that produce a
25 biological factor. In a preferred embodiment the
pharmaceutical composition comprises Sertoli cells and
pancreatic islet cells and a pharmaceutically
acceptable carrier.

 The present invention further provides a
30 compartmentalized kit containing Sertoli cells and
cells that produce a biological factor. An article of

1 manufacture comprising a packaging material and
Sertoli cells contained within the packaging is also
provided.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the glucose responses to oral
sustacal tolerance tests done on the monkey "Lucky" at
intervals before pancreatectomy (Lucky-pre); after
pancreatectomy but prior to transplantations (Lucky-
10 post); and at intervals following transplantation (143
days, 730 days and 930 days, respectively).

Figure 2 shows the C-peptide responses to an
oral sustacal tolerance test at the same time
intervals as depicted in Figure 1.

15 Figure 3 shows the glucose responses to oral
sustacal tolerance tests in the monkey "Oscar".

Figure 4 shows the C-peptide responses in
the same animal and at the same intervals depicted for
Figure 3.

20 Figure 5a and 5b show the effect of
intratesticular islet allografts on serum glucose
levels and the insulin responses to oral glucose in
spontaneously diabetic BB/Wor dp rats. Figure 5a
shows the plasma glucose (mg/dl) concentrations in
25 response to the oral glucose administration of 2 g/kg
of a 50% glucose solution in three groups of rats:
untreated control Sprague Dawley, transplanted
diabetic BB/Wor dp, and insulin treated diabetic
BB/Wor dp rats. Figure 5b shows the serum insulin
30 levels in response to the same dose of oral glucose in

1 untreated control Sprague Dawley, and in transplanted
BB/Wor dp rats.

Figures 6a and 6b show the effect of
intratesticular islet allografts on plasma glucagon
5 secretory responses to oral glucose and a combination
of glucose plus glipizide in spontaneously diabetic
BB/Wor dp rats. Figure 6a shows the plasma glucagon
responses to the oral administration of 2 g/kg of a
50% glucose solution in three groups of rats:
10 untreated control Sprague Dawley, transplanted
diabetic BB/Wor dp, and insulin treated diabetic
BB/Wor dp rats. Figure 6b shows the plasma glucagon
responses to the oral administration of 7 mg/kg of
glipizide and 2 g/kg of a 50% glucose solution,
15 administered 30 minutes later, in three groups of
rats: untreated control Sprague Dawley, transplanted
diabetic BB/Wor dp, and insulin treated diabetic
BB/Wor dp rats. Data points are mean \pm SE of eight
animals in each group.

20 Figure 7 shows a light micrograph of the
pancreatic islets of Langerhans and the isolated rat
Sertoli cells transplanted into the renal subcapsular
space of a diabetic rat.

Figure 8 shows an electron micrograph of an
25 individual cell within the transplanted islet.

Figure 9 shows an electron micrograph of the
fine structure of the extra-islet cells labeled "S" in
Figure 7.

Figure 10 shows the effect of
30 transplantation of piglet islets and Sertoli cells
underneath the renal capsule on the mean daily urine

1 output of seven grafted female rat recipients. Each
bar represents the mean daily urine output over a ten-
day period following transplantation.

Figure 11 shows the effect of the
5 transplantation of piglet islets and Sertoli cells
underneath the skin on the mean daily urine volumes of
three rats over a 50-day period.

Figure 12 shows the light photomicrograph of
pig islets of Langerhans and rat Sertoli cells
10 transplanted into the renal subcapsular space of a
diabetic rat. IL shows the presence of islands of
beta cells (IL) surrounded by an infiltration of small
lymphocytes underneath the renal capsule (K); B (upper
left) shows at higher magnification that the islands
15 (IL) consist of beta cells and B (lower right) shows
that beta cells contain characteristic insulin
granules.

DETAILED DESCRIPTION OF THE INVENTION

20 The present invention is directed to a
method of treating a disease that results from a
deficiency of a biological factor in mammals which
comprises administering to a mammal Sertoli cells and
a therapeutically effective amount of cells that
25 produce the biological factor. As defined by the
present invention, a biological factor is a protein or
nonprotein compound that is necessary for cellular
metabolism and homeostasis. In a preferred
embodiment, the biological factor is a hormone.
30 Hormone producing cells which can be administered
using the method described in the present invention

1 include, for example, pancreatic islet of Langerhans,
pituitary, liver, parathyroid, thyroid and ovarian
cells.

5 In accordance with the present invention,
the Sertoli cells and the cells that produce the
biological factor can be from the same species as the
mammal to be treated or from a different species.
Further, the Sertoli cells and the cells that produce
the biological factor need not be derived from the
10 same species. It has been demonstrated in accordance
with the present invention that Sertoli cells from
pigs in conjunction with islet of Langerhans from pigs
can be used in the treatment of diabetes mellitus in
rats. In a preferred embodiment the Sertoli cells are
15 bovine, porcine or human.

Sertoli cells, which are the predominant
cells of male testes, used in the method described by
the present invention can be separated from other
testicular cells such as Leydig cells, peritubular
20 cells and germ cells, using conventional techniques.
For example, the testes of a male mammal, such as a
boar or ram, are first collected by castration. The
testes are then chopped into several pieces and
subsequently washed by centrifugation.

25 Testicular Leydig cells can be removed from
the tissue suspension using digestion agents such as
trypsin and DNase. The remaining cell suspension is
then washed by centrifugation several times. The
pellet is resuspended in collagenase, incubated and
30 washed by centrifugation to eliminate peritubular
cells within the testes. Testicular germ cells can be

1 removed by incubating the pellet with hyaluronidase
and DNase. After several washings by centrifugation,
the Sertoli cells are collected to transplant using
the method of the present invention.

5 In accordance with the present invention,
the Sertoli cells may be obtained by various
methodologies which establish a line of cells derived
from primary cultures of mammalian Sertoli cells. In
one embodiment the Sertoli cells are immortalized with
10 a chemical or viral transformant, e.g., a temperature-
sensitive mutant of the SV40 virus that allows
propagation and promotes differentiation of the cells.
In another embodiment Sertoli cells are isolated from
mammalian tissue by conventional means using various
15 hydrolytic enzymes such as collagenase, hyaluronidase,
and the like. The cells are further isolated from the
tissue by such conventional methods as filtering and
centrifugation to obtain a purified Sertoli cell
population. The isolated and purified Sertoli cells
20 are next incubated and conventionally immortalized
under conditions known in the art such as treating
said cells with a chemical, that transforms the DNA
thereof, e.g. a mutagen. Examples include N-
nitrosylmethylureas, nitrous acid, hypoxanthine,
25 nitrosamines (see, Freshney, I.R. in Culture of Animal
Cells, A Manual of Basic Technique, 3 ed., Chapter 15,
Wiley-Liss, New York). Alternately, the isolated
purified sertoli cells are incubated in a virus-
containing medium consisting of, e.g., SV40 virus or
30 polyoma virus and a conventional growth medium such as
F12/DMEM, for sufficient time to propagate the Sertoli

1 cells, which are then isolated from the virus. If an
infectious virus cell is being utilized, then it is
preferred that the virus be attenuated by techniques
known in the art. The Sertoli cells may be isolated
5 from the virus or chemical by conventional techniques
employing hydrolytic enzymes. To verify that the
Sertoli cells are produced by this methodology, the
isolated Sertoli cells are optionally screened for the
expression of an appropriate isolate for cloning,
10 e.g., on the basis of expression of mRNAs encoding
Sertoli cell-secreted proteins.

In accordance with the present invention, a
biological factor is a protein or nonprotein compound
that is absent, deficient or altered in a disease
15 state. Cells that produce a biological factor can be
isolated, for example, by first surgically removing
the tissue that produces the factor from a mammal.
This tissue is subsequently chopped and digested using
conventional techniques. For example, the tissue can
20 be digested using a collagenase digestion. The
particular factor producing cells can subsequently be
collected from the digestion mixture using a
separation gradient such as a Ficoll gradient. The
factor producing cells are then grown in tissue
25 culture in serum using conventional techniques.

In accordance with the present invention,
the factor producing cells may be co-cultured with
Sertoli cells in tissue culture. Furthermore, factor-
producing mammalian cells may be co-cultured, co-
30 localized or co-transplanted with Sertoli cells to
enhance the maturation, proliferation and functional

1 capacity of the mammalian cells. It has been
demonstrated in accordance with the present invention
that the maturation of porcine islet cells was
enhanced when these cells were co-cultured with
5 Sertoli cells as evidenced by both the structural
integrity and functionality of the porcine islet cells
compared to the islet cells cultured without Sertoli
cells. Thus, maturation is defined by the present
invention as the process by which a cell develops and
10 becomes functional. The enhanced proliferation of
porcine islet cells co-cultured with Sertoli cells is
evidenced by the larger number of viable, insulin
producing cells compared to porcine islet cells
cultured without Sertoli cells. Proliferation as used
15 herein, is defined as a process in which cells
multiply. The enhanced functional capacity of porcine
islet cells cultured with Sertoli cells is evidenced
by the greater capacity of the co-cultured islet cells
to respond to glucose and glucose plus Forskolin as
20 insulin secretagogues. Functional capacity is defined
as the ability of a cell to respond the biological
environment and to generate various chemical and
biological substances in response to the various
substances present in the biological environment (e.g.
25 when islet cells produce insulin in the presence of
glucose).

Mammalian cells which can be co-cultured,
co-localized or co-transplanted with Sertoli cells as
described by the present invention include, for
30 example, germ cells, such as sperm cells, oocytes,
ovarian cells and zygotes; endocrine cells, such as

1 pancreatic islet cells, chromaffin, thyroid cells,
hepatocytes, parathyroid cells, Leydig cells,
follicular cells; hybridoma cells; recombinantly
transformed cells; epithelial cells; nerve cells and
5 epidermal cells. In a preferred embodiment, the
mammalian cell is a germ cell or endocrine cell.
Cells grown in tissue culture can be transplanted into
a mammal in conjunction with the Sertoli cells using
the methods of the present invention. In accordance
10 with the present invention, factor producing cells may
be stored using a variety of conventional techniques,
such as cryopreserving the cells prior to growth in
tissue culture for subsequent transplantation. It has
been observed in accordance with the present
15 invention, that Sertoli cells co-cultured, co-
localized or co-transplanted with mammalian cells, and
in particular factor producing cells such as islet
cells, enhance the recovery rate and viability of the
mammalian cells in tissue culture and in particular,
20 enhance the recovery rate and viability of cells that
have been previously stored using techniques such as
cryopreservation. Moreover, when co-localized with
factor producing cells, such as islet cells, Sertoli
cells provide immunoprotection and nutritional support
25 when the two cell types are proximally located.
Sertoli cells protect the factor producing cells, such
as islets, from, inter alia, macrophages, proteins,
lymphokines (e.g., IL-1) and toxic factors released by
activated lymphocytes. Sertoli cells provide
30 nutritional support for islets through Sertoli
secreted growth factors, e.g., IGF (insulin-like

1 growth factor), EGF (epidermal growth factor) and
transferrin, thereby permitting the factor-producing
cells to survive longer than when the Sertoli cells
are not present.

5 In a preferred embodiment the factor is a
hormone, and the hormone producing cells are isolated
from a tissue source as described above. For example,
insulin-producing cells are isolated from the
pancreas. In another preferred embodiment, the factor
10 producing cells are provided by transforming suitable
host cells with a nucleic acid capable of expressing
the factor of interest. Transformed cells are
provided by methods known to one of ordinary skill in
the art, and can be found in a myriad of textbooks and
15 laboratory mammals, including Sambrook et al. (1989)
Molecular Cloning: A Laboratory Manual, Cold Spring
Harbor Laboratories, Cold Spring, New York. If
necessary, the nucleic acid encoding the factor of
interest can be adapted by methods known to one of
20 ordinary skill in the art to effect secretion of the
factor from the transformed cell. The utilization of
Sertoli cells in conjunction with the factor producing
cells in accordance with the method of the present
invention allows the production of an immunologically
25 privileged site and production of cell stimulatory
factors in the treated mammal.

The administration of factor producing cells
and Sertoli cells into a mammal is accomplished by
conventional techniques. In a preferred embodiment,
30 administration is by transplantation and the factor
producing cells are injected into the mammal

1 concurrently with or immediately after the injection
of the Sertoli cells into the same site. In another
embodiment, Sertoli cells and cells producing the
biological factor are co-localized and administered to
5 a mammal. As an example, islets and Sertoli cells are
co-encapsulated and injected into the mammal
intraperitoneally. In another embodiment, co-
localized islets and Sertoli cells are transplanted,
injected or provided into a biological or non-
10 biological biocompatible material (biomaterial).
Examples of a biological biocompatible material
include an isolated segment of small intestine with
intact circulation, a pouch (e.g. an omental pouch or
a gastric pouch etc.), a biocompatible polymeric
15 scaffold, a polymeric sponge or matrix, and the like,
prepared pursuant to conventional techniques. On the
other hand, a non-biological, biocompatible material
includes reticulated thermoplastics such as
acrylonitrile vinyl chloride copolymer (PAN-PVC), and
20 the like. The biomaterial may be conventionally
implanted in a mammal. In accordance with the present
invention, an exogenous biological factor may be
administered following the transplantation of factor
producing cells and Sertoli cells until the
25 transplanted cells produce a therapeutically effective
amount of the biological factor. For the treatment of
diabetes, for example, insulin may be administered
following the transplantation of pancreatic islet
cells and Sertoli cells until the transplanted islet
30 cells produce a therapeutically effective amount of
insulin.

1 The Sertoli cells and factor producing cells
of the present invention can be transplanted or co-
localized using any technique capable of introducing
the cells into the mammal such as parenteral
5 administration, subcutaneous administration following
surgical exposure to a desired site, biocompatible
scaffold, sponge or matrix delivery or intraperitoneal
administration. Prior to transplantation, the
recipient mammal is anesthetized using local or
10 general anesthesia according to conventional
technique. In a preferred embodiment the mammal to be
treated is human. In another embodiment the present
method of treating disease further comprises
administering an immunosuppressive agent such as, for
15 example, cyclosporine, tacrolimus, desfergualin and
monoclonal antibodies to, e.g., T cells. In a
preferred embodiment the immunosuppressive agent is
cyclosporine. In another preferred embodiment
cyclosporine is administered at a dosage of from 0.5
20 mg to 200 mg/kg body weight. In a most preferred
embodiment cyclosporine is administered at a dosage of
from 5 mg to 40 mg/kg body weight.

It has been discovered in accordance with
the present invention that administration of Sertoli
25 cells results in the creation of an immunologically
privileged site in the treated mammal and in the
production of cell stimulatory factors. An
immunologically privileged site as defined by the
present invention is a site in the mammal where the
30 immune response produced in response to the
transplanted cells is suppressed due to immuno-

1 suppressive agents produced by Sertoli cells.
Immunologically privileged sites are characterized by
an available blood supply to provide nourishment for
the transplanted cells and a dense tissue to keep the
5 transplanted cells within close proximity of each
other. Examples of immunologically privileged sites
as defined by the present invention include the renal
subcapsular space, subcutaneous facie, the brain and
the hepatic portal vein. Cell stimulatory factors are
10 defined by the present invention as factors that
enhance the viability of mammalian cells. For
example, it has been shown in accordance with the
present invention that Sertoli cells increase the rate
at which the transplanted factor producing cells
15 vascularize in the transplanted site (i.e. promote
angiogenesis). Further, it has been shown by the
present invention that these cell stimulatory factors
enhance the maturation, proliferation and functional
capacity of cells transplanted with Sertoli cells. It
20 is therefore indicated that the Sertoli cells produce
cell stimulatory factors which enhance of viability of
mammalian cells as evidence, for example, by the
increased vascularization rate of the transplanted
islet cells. As used herein, viability denotes the
25 number of living cells in a preparation.

In a preferred embodiment, the present
invention describes a method of treating diabetes
mellitus by transplanting islet of Langerhans in
conjunction with Sertoli cells to create an
30 immunologically privileged site. Allografts as used
in the present invention describes the transfer of

1 tissues or cells between two genetically dissimilar
mammals of the same species. The term xenografts in
the present invention describes the transfer of
tissues or cells between two mammals of different
5 species.

The transplanted islet of Langerhans cells
and Sertoli cells used in the method described by the
present invention can be prepared using any number of
conventional techniques. For example, islet of
10 Langerhans cells can be prepared from the pancreas of
several mammals of the same species. The pancreases
are pooled together, chopped up and digested using
collagenase. The islet of Langerhans cells can be
further isolated using conventional gradients. Once
15 isolated, the islet cells can be grown in culture and
then transplanted in conjunction with Sertoli cells to
create an immunoprivileged site.

Sertoli cells used in the method described
by the present invention can be derived from primary
20 cultures of mammalian Sertoli cells according to the
methods known to one skilled in the art including the
method of e.g. Roberts et al. (1995) Biology of
Reprod. 53:1446-1453, the contents of which is
incorporated herein by reference, or the Sertoli cells
25 can be isolated from mammalian male testes. To
collect the islet cells, the testes are first chopped
into several pieces and then washed by centrifugation.
Leydig cells, present in the crude mixture, can be
removed from the tissue suspension using digestion
30 agents such as trypsin and DNase. The remaining cell
suspension is then washed by centrifugation several

1 times. Following, the pellet may be resuspended in
collagenase, incubated and washed by centrifugation to
eliminate peritubular cells within the testes.
Testicular germ cells can be removed by incubating the
5 pellet with hyaluronidase and DNase. After several
washings by centrifugation, the Sertoli cells for
transplantation can be collected.

The Sertoli cells can be transplanted to
create an immunoprivileged site within a mammal using
10 a variety of techniques. For example, after the
mammal is anesthetized, the Sertoli cells can be
injected into a tissue mass, thereby creating an
immunoprivileged site. The Sertoli cells and factor
producing cells of the present invention can be
15 combined using the techniques capable of co-localizing
the cells such as microencapsulation inside
biocompatible membranes, hydrogels, or reticulated
thermoplastics, for example. The co-localized cells
can subsequently be injected, transplanted or provided
20 into a tissue mass subcutaneously or into a pouch,
e.g. an intestinal pouch, an omental pouch, a gastric
pouch, or a biocompatible polymeric scaffold, sponge
or matrix consisting of, e.g., polylactic acid. Once
injected, transplanted, or provided, the co-localized
25 product is used to treat diseases caused by a
deficiency of the biological factor. For example, the
Sertoli cells and islet cells in combination are
useful in treating diabetes mellitus.

Sertoli cells are administered in an amount
30 effective to provide an immunologically privileged
site. Such an effective amount is defined as that

1 which prevents immune rejection of the subsequently or
co-administered cells that produce the biological
factor. Immune rejection can be determined for
example histologically, or by functional assessment of
5 the factor produced by the cells.

The present invention further provides a
method of creating systemic tolerance to a subsequent
transplant in a mammal by transplanting Sertoli cells
prior to said subsequent transplant as described
10 herein. A transplant as used herein is a mammalian
cell, tissue, organelle or organ that is removed from
one mammal and placed in the same or different mammal.
The subsequent transplant may be made in the same site
or a secondary site. A secondary site as used herein,
15 is a transplantation site in the mammal different from
the initial transplantation site. Systemic tolerance
is demonstrated by various biological phenomena. For
example, systemic tolerance results in a diminished
destructive immune response to a subsequent allograft
20 or xenograft in a mammal without the administration of
prolonged immunosuppressive agents or the co-
transplantation of Sertoli cells. In accordance with
the present invention, the allograft or xenograft may
be any type of transplant, including cells, tissues,
25 organelles or an organ. The types of cells which may
be transplanted in accordance with the methods
described by the present invention include, for
example, endocrine cells, bone marrow cells,
hepatocytes or liver cells, nerve cells or brain
30 cells, and islet cells (fetal, neonatal or adult).
The types of tissues, organelles or organs which may

1 be transplanted in accordance with the methods
described by the present invention include, for
example, heart, kidney, pancreas, liver, skin,
ligaments, tendons and cartilage.

5 As demonstrated by the present invention, a
mammal may be tolerized (i.e. systemic tolerance may
be achieved) by a variety of procedures. For example,
systemic tolerance may be achieved by transplanting an
allograft or xenograft with Sertoli cells and
10 subsequently transplanting the same type of allograft
or xenograft without Sertoli cells or a prolonged
administration of immunosuppressive agents. Systemic
tolerance may also be achieved by transplanting an
allograft of any cell, tissue, organelle or organ
15 without Sertoli cells or prolonged immunosuppressive
agents following an initial transplantation of an
allograft with Sertoli cells. In a preferred
embodiment Sertoli cells are administered in amounts
ranging from 10^1 to 10^{10} cells. In a more preferred
20 embodiment, 10^5 to 10^{10} cells are administered.

The cells producing the biological factor
are administered in a therapeutically effective
amount. The ordinary skilled artisan can determine
the appropriate amount of cells producing the
25 biological factor by methods known in the art. The
amount of cells is dependent upon the amount of factor
being produced by the cells and the known
therapeutically effective amount of the factor
necessary to treat the disease. For example, 1 to
30 1000 islet cells per gram body weight can be
administered to treat diabetes using allografts, 20 to

1 1000 islets per gram body weight are administered
using xenografts. In another preferred embodiment, 5
to 100 islet cells per gram body weight are
administered to treat diabetes. In a most preferred
5 embodiment, 5 to 20 islet cells per gram body weight
are administered, using allografts and 100-1000 islet
cells per gram body weight are administered for
xenografts.

In another embodiment the present method of
10 treating diabetes further comprises administering an
immunosuppressive agent such as, for example,
cyclosporine, tacrolimus, desfergualin and monoclonal
antibodies to, e.g., T cells. In a preferred
embodiment the immunosuppressive agent is
15 cyclosporine. In another preferred embodiment
cyclosporine is administered at a dosage of from 0.5
mg to 200 mg/kg body weight. In a most preferred
embodiment cyclosporine is administered at a dosage of
from 5 mg to 40 mg/kg body weight.

20 More generally, the immunosuppressive agent
can be administered for a time sufficient to permit
the transplanted islets to be functional. This period
extends from the point prior to or immediately
following the transplantation of the islets to the
25 point at which the cells are capable of producing
therapeutically effective amounts of insulin. In a
preferred embodiment, the sufficient period of time to
administer an immunosuppressive agent is about 40 to
about 100 days following transplantation of the
30 islets. In a more preferred embodiment, the
sufficient period of time is about 50-60 days.

1 A preferred embodiment of this invention is
directed to a method of treating Type I and Type II
diabetes mellitus by transplanting islet of Langerhans
in conjunction with Sertoli cells into the renal
5 subcapsular space.

Unlike the therapies for diabetes described
in the prior art, the method of treating diabetes
described by the present invention prevents the
complications of the disease process and does not
10 result in the adverse side effects associated with
conventional diabetes therapy. Furthermore, the
method of transplanting islet cells described by the
present invention provides the necessary factors for
angiogenesis, growth enhancing and increased
15 functional capacity of the islet transplants.

A method of creating an immunologically
privileged site in a mammal is further described by
the present invention. An immunologically privileged
site is created by transplanting isolated Sertoli
20 cells into a mammal in an amount effective to create
an immunologically privileged site. In a preferred
embodiment, 10^1 to 10^{10} cells are administered. In a
more preferred embodiment, 10^5 to 10^{10} cells are
administered. In a preferred embodiment the Sertoli
25 cells are transplanted into the renal subcapsular
space or subcutaneous facie by injection. In a
preferred embodiment the mammal is a human and the
Sertoli cells are human or porcine.

A further aspect of the present invention is
30 directed to a method of enhancing the recovery rate
and viability of frozen mammalian cells in tissue

1 culture comprising co-culturing the frozen mammalian
cell with Sertoli cells. As shown in accordance with
the present invention, Sertoli cells produce cell
stimulatory factors which enhance the recovery rate
5 and viability of mammalian cells previously frozen.
Mammalian cells may be frozen using a widely
conventional techniques, including, for example,
cryopreservation.

Further contemplated in accordance with the
10 present invention is a method of enhancing the
recovery and proliferation of ex vivo cells comprising
co-culturing said cells with Sertoli cells for a time
and under conditions sufficient to achieve said
enhanced recovery and proliferation.

15 Another aspect of the present invention
provides a pharmaceutical composition comprising
Sertoli cells and cells producing a biological factor
and a pharmaceutically acceptable carrier. In a
preferred embodiment the composition comprises Sertoli
20 cells and islet of Langerhans cells and a
pharmaceutically acceptable carrier. A further
preferred embodiment of the present invention
comprises using porcine, bovine or human Sertoli cells
and porcine, bovine or human islet of Langerhans
25 cells. As used herein, a pharmaceutically acceptable
carrier includes any and all biological and non-
biological biocompatible membrane materials. A
pharmaceutically acceptable carrier also includes any
conventional solvents, dispersion media, coatings,
30 antibacterial and antifungal agents, isotonic agents

1 and the like. The use of such media and agents is well-known in the art.

The present invention further contemplates a pharmaceutical composition comprising Sertoli cells
5 and a pharmaceutically acceptable carrier. This pharmaceutical composition, upon administration to a mammal, can be used to treat a variety of diseases, such as for example, autoimmune diseases. Accordingly, the present invention is further directed
10 to treating an autoimmune disease in a mammal comprising administering a therapeutically effective amount of Sertoli cells to the mammal.

The present invention is further directed to a method of enhancing the recovery and proliferation
15 of ex vivo cells comprising culturing said cells with a culture media from a tissue culture containing Sertoli cells for a time and under conditions sufficient to achieve said enhanced recovery and proliferation. As contemplated by the present
20 invention, Sertoli cells are cultured using a conventional tissue culture media as described herein for a time and under conditions sufficient for the Sertoli cells to produce, for example, cell stimulatory factors. The Sertoli cells are then
25 removed from the culture media and the culture media is used in subsequent tissue cultures, for example, as a culture media for sperm cells previously stored by cryopreservation.

Another aspect of the present invention is
30 directed to methods of co-localizing biological factor producing cells, e.g., islets of Langerhans, with

1 Sertoli cells to enhance long-term immunoprotection
and nutritional survival of transplanted factor
producing cells, e.g., islets. The methods of co-
localization include co-localization in an intestinal
5 segment, pouch, e.g. an omental pouch, a gastric pouch
or biocompatible polymeric scaffold, sponge or matrix,
for example. The method of co-localization in an
intestinal segment comprises:

- (a) isolating a segment of mammalian small
10 intestine with intact mucosa and intact circulation;
- (b) removing the mucosal layer of the small
intestine and closing the ends of the isolated
segment;
- (c) implanting biological factor producing
15 cells and Sertoli cells into the isolated segment; and
- (d) fixing the isolated segment to the
small intestine.

Methods of co-localizing biological factor
producing cells, e.g., islets with Sertoli cells in
20 pouches, such as omental or gastric pouches are also
contemplated by the present invention as described by
Amiri, et al. (1990) Arch. Surg. 125:1472-1474 and
Bayat, et al. (1995) Surg. Res. Commun. 17:87-91, both
of which are incorporated herein by reference.

25 Procedures for co-localizing islets and Sertoli cells
in biologically compatible pouches are general and
conventionally employed on a case-by-case basis by the
skilled artisan in accordance with the present
invention.

30 Procedures for co-localizing islets and
Sertoli cells in polymeric scaffolds are readily

- 1 appreciated by the skilled artisan. It is preferred
that the polymeric templates used in accordance with
the present invention are biodegradable and comprise a
polyvinyl alcohol, e.g., poly-L-lactic acid.
- 5 Polyvinyl alcohol based templates provide sufficient
porosity to permit rapid tissue ingrowth and
prevascularization before cell transplantation. In
essence, the polymeric template employed in connection
with the present invention acts as a matrix or sponge
10 permitting the ingrowth of blood vessels and tissue
which facilitates the co-localization of, e.g., islets
and Sertoli cells by providing ready access to a
nutrient-rich blood supply.

- It is preferred that the Sertoli cells are
15 provided in an amount ranging from about 10^1 to about
 10^{10} cells. In a more preferred embodiment, Sertoli
cells are provided in an amount ranging from about 10^4
to about 10^{10} cells. In yet another preferred
embodiment, the factor producing cells are pancreatic
20 islets. The islets are provided in a preferred amount
of about 5 to about 200 cells per gram of body weight,
and in a more preferred amount of about 5 to about 100
cells per gram of body weight.

- A further aspect of the present invention is
25 directed to a method of encapsulating biological
factor producing cells, e.g., islets, with Sertoli
cells to enhance long-term immunoprotection and
nutritional survival of transplanted islets. The
method of encapsulation comprises:

- 30 (a) suspending a pharmaceutically effective
amount of biological factor producing cells, e.g.,

- 1 islets, and Sertoli cells in combination with a
gelling effective amount of a first water soluble
gelling agent in an aqueous medium which is
physiologically compatible with the cells and
5 extruding the islet/Sertoli cell/gelling agent mixture
to form a droplet containing the islets and Sertoli
cells;
- (b) subjecting the product of step (a) to
an effective amount of network forming cations to form
10 discrete capsules of sufficient size to encapsulate
the islets and Sertoli cells together;
- (c) forming a semipermeable membrane around
the capsules to obtain a single-walled bead
encapsulating the cells; and
15 (d) contacting the single-walled bead with
a gelling effective amount of a second gelling agent
so as to form a second semi-permeable membrane
encapsulating the product of step (c).

The gelling agent may be any water-soluble
20 material which can be gelled to form a bead. A
preferred gelling agent is a water soluble, natural or
synthetic polysaccharide gum such as an alkali metal
alginate. A preferred gum is sodium alginate. Other
gums which may be used include guar gum, gum arabic,
25 carrageenan, pectin, tragacanth gum, xanthan gum or
their acidic fractions.

In a preferred embodiment, the Sertoli cells
and factor producing cells are encapsulated within an
alginate polylysine-alginate semi-permeable hydrogel.
30 It is preferred that the Sertoli cells are derived
from bovine, porcine, and human sources and are

1 produced by a cell line in accordance with the present
invention. It is preferred that the Sertoli cells are
provided in an amount of from 10^1 to 10^{10} cells. In a
more preferred embodiment, Sertoli cells are provided
5 in an amount of from about 10^4 to 10^{10} cells. In yet
another preferred embodiment the factor producing
cells are pancreatic islets. The islets are provided
in a preferred amount of about 5 to about 200 cells
per gram of body weight, and in a more preferred
10 amount of about 5 to about 100 cells per gram of body
weight.

The procedure for the encapsulating of
Sertoli cells with cells that produce a biological
factor is general, and the procedure will be explained
15 in more detail with respect to Sertoli cells and islet
cells, which are exemplary.

In an embodiment of step (a) of the subject
method, the first gelling agent is sodium alginate.
It is conventionally suspended in an aqueous solution
20 such as a buffer or saline solution containing the
islets and Sertoli cells.

By "gelling effective amount" is meant an
amount of a water soluble gelling agent capable of
binding calcium ions or other ions that interact with
25 the gelling agent to form a network. More
specifically, the islets and Sertoli cells in
combination are preferably suspended relative to the
gelling agent in a ratio of about 1:20 to 20:1 (v/v)
and more preferably 1:10 to 10:1 (v/v) and most
30 preferably about 1:10 (v/v). Preferably, the Sertoli

- 1 cells and islets cells are present in a ratio of about
1:1 (v/v).

The suspension is extruded by techniques commonly used in the art. It is preferred that the
5 suspension is extruded through an air-jet needle. In a preferred embodiment, droplets containing islets and Sertoli cells in association with the alginate are produced by extrusion (1.7 ml/min) through a 22 gauge air-jet needle (air flow 5 l/min).

- 10 In an embodiment of step (b) of the subject method, the droplets are subjected to a solution of multivalent cations, such as a solution of calcium salt, e.g., calcium halide, e.g., calcium chloride solution, which form a network within said droplet.
15 The preferred concentration is at least about 0.5% (v/v), and more preferably at least about 1% (v/v), and most preferably ranging from about 1% (v/v) to a saturated solution. In an embodiment, the droplets fall into a beaker containing a saline solution at pH7
20 of calcium chloride solution, e.g., 10 ml 1.1% CaCl₂ in 0.9% saline at pH7. This process continues for a sufficient time until the negatively charged alginate droplets bind calcium and form calcium alginate gel.

- In an embodiment of step (c) of the subject
25 method, a membrane is formed around the product of step (b) by subjecting the encapsulated product to polymers, which polymers contain substituents reactive with the gelling agents, especially the acid groups of the gelling agent. The preferred polymers are
30 polyamine acids such as poly-L-lysine (PLL) or polyethylenimine. In a preferred embodiment, the

1 polymer is poly-L-lysine with a molecular weight of
about 20kd. It is preferred that the polymers coat
the product of (b) by following the procedure of
Goosen, et al. in Biotech. Bioeng., 27: 146-150
5 (1985), the contents of which is incorporated herein
by reference. Without wishing to be bound, it is
believed that positively charged poly-L-lysine
displaces calcium ions and binds negatively charged
alginate, producing a polyelectrolyte membrane.

10 In an embodiment of step (d) of the subject
method the second gelling agent may be sodium alginate
which may improve the biocompatibility of the capsule
in a mammal. The second gelling agent may be added
according to the methods employed by Weber, et al.
15 U.S. Patent No. 5,227,298, incorporated herein by
reference. The double walled semi-permeable capsules
formulated in connection with the present invention
have molecular weight cut-offs in the range of 50,000
daltons and provide sustained release of factors
20 produced by the encapsulated islets and Sertoli cells.
The capsules comprise semi-permeable membranes which
function to protect islets from immune responses while
simultaneously permitting passage of biological
factors produced by islets into the mammal. In still
25 another embodiment of the present invention, co-
localized, e.g., co-encapsulated islets and Sertoli
cells are connected to a blood supply by techniques
known in the art, thereby permitting the free flow of
nutrients and inhibiting the influx of molecules
30 produced by the immune system.

1 The co-localized, e.g., encapsulated cells
producing biological factor and Sertoli cells are
effective in treating a disease resulting from a
deficiency of said biological factor. For example,
5 the co-localized, e.g., encapsulated islet cells with
Sertoli cells, are effective in treating diabetes
mellitus. Thus a preferred embodiment of this
invention is directed to a method of treating diabetes
mellitus by co-localizing, e.g., co-encapsulating and
10 transplanting islet of Langerhans into the peritoneal
space. This method not only prevents the
complications of the disease process, but also reduces
the adverse effects associated with other therapies.
This method also provides a biological factor in
15 appropriate amounts which are released in a
physiological manner.

 The present invention is also directed to a
kit for treatment of a disease. In one embodiment,
the kit is compartmentalized to receive a first
20 container adapted to contain Sertoli cells in an
amount effective to create an immunologically
privileged site in a mammal, and a second container
adapted to contain a therapeutically effective amount
of cells that produce a biological factor that is
25 absent or defective in the disease to be treated. In
a preferred embodiment, the Sertoli cells are bovine,
porcine or human and are provided in an amount of from
 10^1 to 10^{10} cells. In a more preferred embodiment,
Sertoli cells are provided in an amount of from 10^5 to
30 10^{10} cells. In another preferred embodiment the cells
that produce a biological factor are cells that have

1 been transformed with DNA encoding the factor. In yet
another preferred embodiment the cells that produce
the factor are pancreatic islet cells. The islet
cells are provided in a preferred amount of 5 to 200
5 cells per gram of body weight, and in a more preferred
amount of 5 to 100 cells per gram of body weight.

The present invention further provides an
article of manufacture comprising a packaging material
and Sertoli cells contained within said packaging
10 material, wherein said Sertoli cells are effective for
creating an immunologically privileged site in a
mammal, and wherein said packaging material contains a
label that indicates that said Sertoli cells can be
used for creating an immunologically privileged site
15 in a mammal. The packaging material used to contain
the Sertoli cells can comprise glass, plastic, metal
or any other suitably inert material.

Unless specified to the contrary, it is to
be understood that percentages are by volume.

20 In order to further illustrate the present
invention, the experiments described in the following
examples were carried out. It should be understood
that the invention is not limited to the specific
examples or the details described therein. The
25 results obtained from the experiments described in the
examples are shown in the accompanying figures and
tables.

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EXAMPLE 1

Six male Rhesus monkeys were transplanted with islet allografts in their testes to examine the survival of these transplants. The recipients were made diabetic by means of a near total pancreatectomy, followed two weeks later by an intravenous injection of 35 mg streptozotocin/kg body weight. This procedure resulted in the induction of severe diabetes melitis. Plasma glucose levels were in excess of 400 mg/dl and the animals were ketotic. Malabsorption was prevented by the oral administration of VACUOUS®, one tablet given twice daily before each meal.

Islets were isolated from female Rhesus monkeys. First, the pancreases of five animals were removed, pooled and chopped finely into smaller fragments. After collagenase digestion in a water bath at 37°C, the islets were separated from exocrine tissues and other cellular debris on at least two Ficoll gradients, prepared in tandem. The islets were washed three times by centrifugation in ice-cold Hanks's buffer and then handpicked and transferred in groups of 150 to biologic grade Petri dishes. Each dish contained 6 mL of culture medium CMRL-1066 supplemented with 5% fetal calf serum, glucose at a concentration of 250 mg/dL, penicillin (100 U/mL), and streptomycin (100 µ/mL). Incubation of islets were carried out at 35°C in 5% CO and air for 4 to 6 days. The islets were transferred to fresh medium at 48 hour intervals.

1 Viability and counting of the islets were
facilitated by means of the uptake of the dye
dithizone. Each monkey received an average of about
10⁴ islets/kg body weight injected into both testes.
5 In the first three animals the testes were elevated
into the abdominal cavity, whereas in the last three
recipients the grafted organs were anchored into the
inguinal canal. Cyclosporine (CsA) was administered,
in varying doses to the first three grafted animals
10 over a 30 day period, whereas the last three hosts
were given 7 injections of CsA (20 mg/kg) on days -4
to +3. Oral sustacal tolerance tests were done on day
30, and then at intervals in the normoglycemic
animals, as follows.

15 The monkeys were housed individually in
cages and given standard monkey chow and fruit twice
daily. In addition, a pancreatic enzyme was mixed
with the food since the monkeys had been
pancreatectomized to make them diabetic before
20 transplantation.

 The night before the test, the animals were
fasted for 12 hours. At 8 a.m. the next morning they
were then anesthetized and prepared for the test meal.
Sustacal was used as the test agent. Sustacal
25 consists of a physiologic mixture of carbohydrates,
proteins and fat which closely mimics a standard meal
and which is a powerful stimulus for the release of
insulin.

 Sustacal was injected directly into the
30 stomach of the sleeping animal through a nasogastric
tube. Blood samples were then obtained at times 0,

1 15, 30, 60, 90, 120 and 180 minutes. The samples were
centrifuged and the serum stored at -20°C until
measurements for insulin or C-peptide could be carried
out. C-peptide is a very sensitive marker for beta
5 cell function. The results are shown in Figures 1-4.

Figure 1 shows the glucose responses to oral
sustacal tolerance tests done on the monkey "Lucky" at
intervals before pancreatectomy (Lucky-pre); after
pancreatectomy but prior to transplantation (Lucky-
10 post); and at intervals following transplantation (143
days, 730 days and 930 days, respectively).

It can be readily appreciated that the
animal became severely diabetic after the removal of
his pancreas (Lucky-post). Following transplantation
15 the glucose responses were restored to normal levels
at all of the time intervals measured (143, 730 and
930 days following transplantation). Lucky showed no
evidence of graft failure. With graft failure glucose
levels would become elevated would approach those
20 which were found following his pancreatectomy.

Figure 2 shows the C-peptide responses to an
oral sustacal tolerance test at the same time
intervals as depicted in Figure 1. Following his
pancreatectomy the C-peptide responses became blunted
25 indicating a severe diabetes. But following
transplantation the levels were not only restored to
normal but appeared to show a hyperresponsive pattern
of C-peptide release and levels done on day 730 exceed
the normal levels at all points measured. The
30 elevated levels might be due to the fact that insulin
released from the testis enters the systemic

1 circulation. By contrast, insulin released from the
pancreas enters the portal vein and travels
immediately to the liver where about 60% is broken
down during the first passage. Insulin released into
5 the systemic circulation reaches the liver much later,
thus the elevated levels. As was evident with an
investigation of the glucose concentrations, the C--
peptide responses showed no evidence of failure 30
months following transplantation.

10 Figure 3 shows the glucose responses to oral
sustacal tolerance tests in the monkey "Oscar".
Following the removal of his pancreas he became
severely diabetic with elevated glucose levels.
Following transplantation of islets the glucose
15 responses became similar to those determined before
his pancreas was removed. The glucose levels remain
within normal levels 32 months following
transplantation.

 Figure 4 shows the C-peptide responses in
20 the same animal and at the same intervals depicted for
Figure 3. The animal became very diabetic following
the removal of his pancreas and shows blunted
C-peptide responses as a result. Following
transplantation and for the next 730 days the C-
25 peptide responses were greater compared with the
normals. on day 930 following transplantation the C-
peptide responses have become somewhat less compared
with the normals. Despite somewhat lower C-peptide
levels the animal remains normoglycemic.

30 This example demonstrates that primates can
be successfully transplanted with intratesticular

1 islet allografts without the need for sustained
immunosuppression, and that functional integrity of
intratesticular islet allografts is maintained for
periods exceeding two years with no evidence of graft
5 failure.

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EXAMPLE 2

This study examined insulin and glucagon secretory patterns in spontaneously diabetic bb/Wor dp rats transplanted with abdominal, intratesticular, islet grafts. Diabetic, BB/Wor dp, rats received intratesticular islet grafts from MHC-compatible BB/Wor dr rats and no immunosuppression. After a period of 74±15 days, of normoglycemia, three different groups (controls; BB/Wor dp, transplanted; and BB/Wor dp, insulin treated) were given the following challenges; (1) an oral glucose tolerance test (OGTT), (2) a single oral dose of glipizide, followed by an OGTT, and (3) arginine, by intravenous infusion. The results of this study are shown in Tables 1 and 2 and Figures 5 and 6.

TABLE 1

Metabolic Parameters and Immunoreactive Serum Insulin and Glucagon Levels in Control and in Transplanted and Insulin Treated BB/Wor dp Rats

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	BB/Wor dp		
	CONTROLS	GRAFTED*	INSULIN TREATED
Plasma Glucose (mg/dl): Prior to Therapy	112±5	502±8+	510±13+
After 2.5 Months	97±4	110±3	350±40#
Duration p.t. OGTT (days)	75±6	70±11	78±19
Weight Gain (g)	120±6	105±17	48±14\$
Fasting Plasma Insulin (uU/ml)	21.9±3	20.4±2	ND
Fasting Plasma Glucagon (pg/ml)	37.8±5.7	43.4±4.6	47.4±4.9

* Duration of normoglycemia after grafting (days) = 279 ± 25

+ P < 0.0001 vs. control

P < 0.0001 vs. grafted

\$ P < 0.02 vs. grafted

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TABLE 2

Pancreatic and Testicular Insulin
and Glucagon Content in Control and in
Transplanted and Insulin Treated BB/Wor dp Rats

	BB/Wor dp		
	CONTROLS	GRAFTED	INSULIN TREATED
Pancreas (mg)	1573±171	757±122	920±32
Insulin (ug/g)	66±5.03	0.58±0.18	0.76±0.12
Glucagon (ng/mg)	4.1±0.35*	49.±0.33**	6.9±0.08
Testes Fractions: (mg)	493±49.6	582±59.2	430±28.0
Insulin (ug/g)	0.0	59.70±0.49	0.0
Glucagon (ng/mg)	0.0	1.4±0.37	0.0

* P < 0.03

** P < 0.08 vs. diabetic, respectively

Figure 5 shows the effect of intratesticular islet allografts on serum glucose and insulin responses to oral glucose in spontaneously diabetic BB/Wor dp rats. Figure 6 shows the effect of intratesticular islet allografts on plasma glucagon secretory responses to oral glucose and a combination of glucose plus glipizide in spontaneously diabetic BB/Wor dp rats. This experiment demonstrates that grafted testes in spontaneously diabetic BB/Wor dp rats contain both alpha and beta cells, and that the alpha and beta cells have the capacity to respond to specific secretagogues independently.

EXAMPLE 3

This study investigated the effect of Sertoli cell enriched fraction (SEF) on islet allograft survival in the renal subcapsular space of diabetic rats.

The animals used in this study were PVG rats, weighing between 150-200 g. Diabetes was induced by means of a single intravenous injection of 65 mg/dL of streptozotocin. Only rats with plasma glucose levels in excess of 400 mg/dL were transplanted. Sprague Dawley (S-D) outbred rats were used as islet donors. Either PVG or S-D male rats between 16 and 18 days old were used as Sertoli cell donors.

Islet Preparation

Islets were prepared according to modification of the method of London et al. (1990) Transplantation, 49: 1109-1113. The islets were purified on Ficoll gradients, and the isolated cells were then incubated for 4 days at 37°C in a humidified atmosphere of 5% CO₂, and air prior to use. No special efforts were made to deplete the islets of contaminating passenger leukocytes.

Sertoli Cell-enriched Fraction Preparation

Highly purified preparations of Sertoli cells were isolated from the testes of young males according to the method of Cheng et al. J. Biol. Chem., 26:12768-12779. The testes were removed, chopped into several pieces, and placed in a 50 mL

- 1 conical tube containing 50 mL of Ham's F12/DMEM media.
The pieces were washed once by centrifugation at 800 x
g for 2 min. The supernatant was aspirated, and the
tissue resuspended in 40 mL of media containing 40 mg
- 5 trypsin and 0.8 mg DNase in a sterile 250 mL
Erlenmeyer flask. The flask was placed in 37°C
oscillating incubator at 60-90 osc/min for 30 min.
This step removed Leydig cells. The tubules were then
transferred to a 50 mL conical tube, and centrifuged
- 10 at 800 x g for 2 min. The supernatant fraction was
aspirated, and the pellet resuspended in 40 mL of 1 M
glycine, 2 mM EDTA containing 0.01% soy bean trypsin
inhibitor and 0.8 mg DNase, and incubated at room
temperature for 10 min. This step lysed any residual
- 15 Leydig cells. The cells were washed by centrifugation
for 2 min, and the step repeated twice, or until the
media was no longer cloudy. The pellet was
resuspended by gentle homogenization with a glass
Pasteur pipet in 40 mL of media containing 20 mg
- 20 collagenase in an Erlenmeyer flask, and incubated at
37°C for 5 min with 60-90 osc/min. The cell
suspension was centrifuged at 800 x g for two min, and
the pellet resuspended by gentle homogenization with a
Pasteur pipet in 40 mL media containing 40 mg
- 25 collagenase and 0.2 mg DNase, and incubated in an
Erlenmeyer flask at 37°C for 30 min with 60-90
osc/min. The cells were then washed by centrifugation
for 2 min, and the process repeated at least three
times to eliminate peritubular cells. The, cells were
- 30 resuspended by gentle homogenization with a Pasteur
pipet in 40 mL media containing 40 mg hyaluronidase

- 1 and 0.2 mg of DNase, and incubated at 37°C for 30 min
with 60-90 osc/min. The cells were pelleted by soft
centrifugation for 2 min, and washed at least five
times to eliminate germ cells. The resultant SEF was
5 resuspended in 0.25 mL of media, and immediately
transplanted into the recipient rat. Each grafted rat
received the equivalent of the total amount of Sertoli
cells contained in a single testis.

10 Transplantation of Rats

- The diabetic rat was anesthetized with
methoxyflurane USP in a sterile hood and the left
flank opened to expose the kidney. The Sertoli-
enriched fraction containing approximately 5 million
15 Sertoli cells was injected first underneath the renal
capsule. The cells could be seen as a milkish bubble
underneath the capsule. Immediately afterwards, a
total of 10 islets/g of body weight was injected to
the same milkish bubble. The needle was retracted
20 slowly to prevent leakage of the grafted cells.
Cyclosporine (CsA) was administered subcutaneously in
varying doses over a 20-day period to groups two and
four. Because the grafted rats responded similarly
whether the drug was administered over a 20-day, or
25 over a 3-day period, all of the subsequent groups,
including the female rats, were treated with only
three injections of 25 mg/kg CsA, given on days 0, +1,
and +2, relative to the graft. The rats received no
other therapy.

- 30 A total of 36 male and 21 female PVG rats
were divided into six different treatment groups:

1 Group 1, the control group, consisted of 6 male rats
grafted with only islets from S-D donor rats. They
received neither SEF nor CsA. Group 2 consisted of 10
rats grafted with a combination of islets from S-D
5 rats and CsA postransplantation, but no SEF. Group 3
consisted of a total of 10 rats grafted with a
combination of islets from S-D and SEF from PVG donor
rats, but no CsA postransplantation. Group 4
consisted of 10 rats grafted with a combination of
10 islets from S-D donors, SEF from PVG donors, and CsA
postransplantation. Group 5 consisted of 11 female
rats grafted with the same combination of cells as
depicted for Group four. Group 6 consisted of 10
female rats grafted with a combination of islets and
15 SEF, both cell types from S-D donors, and CsA
postransplantation.

Posttransplantation Evaluation of Rats

The grafted rats were transferred to
20 metabolic cages, and plasma glucose levels were
obtained at weekly intervals. Urine volumes and urine
glucose contents were obtained at daily intervals. A
rat was considered cured of the diabetic process if
the following criteria were met: A random plasma
25 glucose level ≤ 150 mg/DL; glycosuria; and immediate
reversal to hyperglycemia following surgical removal
of the grafted kidney.

To determine if any of the rats had become
unresponsive to their grafts, normoglycemic rats were
30 challenged with a secondary islet allograft consisting
of at least 500, freshly prepared, Sprague Dawley

1 islets which were injected into the contralateral
renal subcapsular space. No immunosuppression was
given following the challenge.

To examine the impact of the transplantation
5 of SEF on fertility of the female rats, normoglycemic
animals of longer than 30 days were mated with PVG
males. Metabolic parameters, as outlined above, were
closely monitored, as was the course of their
pregnancies.

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Structural Analysis of Grafted Tissue

A total of five successfully grafted rats
were nephrectomized at intervals following
transplantation. Wedge sections of renal tissue,
15 obtained from sites at which islets and SEF had been
injected, were prepared for examination by light and
electron microscopy, as previously described by
Cameron et al. (1990) Transplantation, 50:649-653.
Briefly, the tissue wedges were immersion-fixed with
20 5% glutaraldehyde in 0.1 M collidine buffer for 1 h,
washed in buffer, and postfixed for 1 h with 1% osmium
tetroxide in 0.1 M buffer. Small tissue blocks were
cut from the wedges, and dehydrated through a graded
series of ethyl alcohols, transferred to propylene
25 oxide, and embedded in Epon 812/Araldite plastic
resin. Thick (0.5 μ m) and thin (900 mg) sections were
stained routinely with toluidine blue and uranyl
acetate/lead citrate, respectively, for structural
analysis by light and electron microscopy. The
30 results are shown in Table 3 and Figs. 7-9.

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TABLE 3

Effect of Sertoli Cells on
Islet Allograft Survival in the
Non-Immunologically Privileged Renal, Subcapsular Site

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Group (n)	Gender	Sertoli Cell (donor origin)	CsA	Duration of Normoglycemia (days) Individual Responses
1 (6)	Male	---	-	0,0,0,0,0,0
2 (10)	Male	---	+	0,0,0,0,0,0,0,130>441, >445
3 (10)	Male	+ (PVG)	-	0,0,0,0,9,10,12,13,13,14
4 (10)	Male	+ (PVG)	+	19,76,58*,84*,167*,127†, 139†,>418†,>422†,>425†
5 (11)	Female	+ (PVG)	+	7,11,14,28,>287†,>305†, >306†,>308†,>441†,>447†, >457†
6 (10)	Female	+ (S-D)	+	8,10,96*,128*,>168,>172, >184,>193,>193,>196

* Nephrectomized

† Challenged with a Secondary Islet Allograft

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Group 1: None of the six rats grafted with islets alone, without either SEF or CsA, became normoglycemic.

25

Group 2: Three of 10 rats grafted with islets and treated with CsA became normoglycemic for more than 100 days. The 3 normoglycemic rats were challenged with a secondary graft on days 116, 192 and 197, respectively. One rat reverted to hyperglycemia on day 130, while 2 remained normoglycemic.

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Group 3: Initially 6 of the 10 rats grafted with islets and SEF, but no CsA, became normoglycemic, but all of them reverted to hyperglycemia by day 14.

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1 Group 4: All 10 of rats grafted with a
combination of SEF and islets, and also given CsA
became normoglycemic. Two reverted spontaneously to
diabetes on days 19 and 76, respectively. Three were
5 nephrectomized on days 58, 84 and 167 following
transplantation. All 3 of these rats became
hyperglycemic within the next 24 h. The remaining 5
rats were challenged with a secondary islet allograft
on days 119, 129, 280 342 and 400, respectively. Of
10 these, the first 2 reverted to diabetes on day 127 and
139, respectively, while the latter 3 remained
normoglycemic.

 Group 5: All 11 of the female rats grafted
with a combination of islets and SEF, and then given
15 CsA, became normoglycemic. of these, 4 reverted
spontaneously to hyperglycemia by day 28. Of the 7
normoglycemic rats who were mated with male PVG rats,
6 became pregnant, and of these, 8 had litters varying
between 1 and 10 pups. They were able to nurse the
20 pups successfully. A total of 7 of the long-term
surviving females were challenged with secondary islet
allografts at least 200 days following
transplantation. None of them reverted to
hyperglycemia.

25 Group 6: of the 10 rats grafted with islets
and SEF from the same donor strain of rat, all 10
became normoglycemic. Two reverted to hyperglycemia
by day 10. A nephrectomy to remove the graft was done
on 2 of the long-term surviving rats on days 96 and
30 201, respectively. Both reverted to hyperglycemic
immediately within the next 24 h.

1 Tissue Morphology

Renal tissue obtained from the long-term grafted kidney appeared structurally normal by light microscopy (Figure 7). Transplanted islets in this organ were immediately subjacent to the kidney capsule, and also appeared structurally normal. They displayed tissue and cellular architecture identical to islets in situ (Figure 7). Individual islet cells were partitioned into cell clusters by thin connective septa containing small vessels and capillaries (Figure 7). It appeared that most of the islet cells contained secretion granules. When resolved by electron microscopy, islet cells were identified as the β -cell type by the inclusion of ultrastructurally distinctive, and unique insulin-containing secretion granules (Figure 8). All β -cell clusters observed were in close proximity to intra-islet capillaries (Figure 8).

There was a high density of cells between, and directly adjacent to, the transplanted islets and renal parenchyma. By light microscopy, they did not appear to be islet cells, kidney cells nor cells of blood origin (Figure 7). When observed by electron microscopy, these cells were similar in ultrastructure to Sertoli cells in that their nuclei were irregular in profile, and contained deep nuclear clefts, distinctive nucleoli were often present, and mitochondrial structure was dense. Although these cells did not retain the typical polarity of Sertoli cells in vivo, they were, however, identical in appearance to Sertoli cells in vitro, when the cells

1 are not plated on a basement membrane substrate. The
cells were not associated with a basement membrane,
and appeared randomly organized (Figure 9). Cells
showing ultrastructural features of either germ or
5 Leydig cells were not observed.

This example demonstrates that an
immunologically privileged site for transplantation of
isolated islet can be created in male and female
diabetic recipients by transplantation of Sertoli
10 cells without the need for sustained
immunosuppression.

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EXAMPLE 4

This study determined the survival of discordant islet xenografts in various nonimmunologically privileged organ sites in experimental animals.

Islets were prepared from young piglets as follows: Male piglets not weighing more than 2.2 kg were used exclusively. The piglet was anesthetized and following exsanguination both pancreas and testes were harvested under sterile conditions. A collagenase solution consisting of 2 mg/ml of collagenase type XI (Sigma) was injected directly into the pancreas. The pancreas was incubated at 37°C for 17 minutes and the digested tissues washed three times by means of centrifugation and aliquots of 1 ml each transferred to Petri dishes. The islets were incubated at 32°C in tissue culture media 199 supplemented with 10% horse serum for six days.

On day seven the cultured islets were collected in batches of $\pm 4,000$ and cryopreserved using a standard protocol. The cells were stored in liquid nitrogen at -196°C for periods varying between two and four weeks. The islets were removed from the liquid nitrogen and thawed using an established procedure. The thawed islets were transferred to Petri dishes and co-cultured with pig Sertoli cells for three days at 32°C in the same 199 culture media as described above. Earlier studies have shown an improved survival rate of thawed islets cultured in the presence of Sertoli cells.

1 On day three following thawing the islets
were hand-picked and counted and a total amount of 12
islets/g of body weight transplanted into female
diabetic Sprague Dawley rats. A total of 5 million
5 Sertoli cells procured from the piglet testes were
grafted simultaneously into the same location. The
organ sites to be tested for the grafting of islets
include: a) the renal subcapsular space, b)
subcutaneously, and c) the liver. Following
10 transplantation, the rats were treated with
cyclosporine as follows: 25 mg/kg for 7 days; 15 mg/kg
for 5 days; 10 mg/kg for 5 days; 5 mg/kg for an
additional 13 days. On day 30 the drug was
discontinued.

15 To demonstrate viability and functional
integrity of isolated piglet islets the following
studies were done: a) staining of Cells with
dithizone, a stain is highly specific for insulin; b)
staining of cells with 0.4% trypan blue which
20 indicates viability of the islets; and c) culturing of
batches of 5 islets in the presence of insulin
secretagogues such as low and high glucose
concentrations at specified intervals following
culturing, cryopreservation and thawing. The results
25 are shown in Table 4.

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TABLE 4

**Insulin Secretion (micro-units/ml) from
Incubated and from Cryopreserved-Thawed Islets
Done on Days 3, 7 and 14 of Culturing, Respectively**

5

	3 DAYS	7 DAYS	14 DAYS
INCUBATED ISLETS PRIOR TO CRYOPRESERVATION:			
a) Low Glucose (90 mg/dl)	15.3±3.8	21.8±1.1	17.29±2.4
b) High Glucose (300 mg/dl)	32.2±5.4	37.14±3.4	23.3±1.8
CRYOPRESERVED AND THAWED ISLETS:			
a) Low Glucose (90 mg/dl)	14.52±2.8	7.13±1.3	5.38±2.02
b) Low Glucose + Sertoli Cells	10.31±2.8	9.17±2.6	8.38±.41

10

TABLE 5

**Yield of Porcine Islets
Following 1, 3 and 7 Days of Culture and the
Percentage of Islets Lost During 7 Days of Culture**

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Pig. No.	BW (kg)	Panc. W g	D1 Islets /g panc.	D3 Islets /g panc.	D7 Islets /g panc.	Islet Loss % D7/D1
1	1.6	1.79	36,536	31,659	27,212	26%
2	2.0	1.89	37,272	32,962	27,883	25%
3	2.3	2.46	29,268	26,046	20,884	29%
4	1.8	1.66	39,904	37,726	31,664	21%
5	1.8	1.76	37,846	34,578	30,046	21%
6	1.6	1.74	39,866	37,888	32,424	19%
7	1.4	1.61	42,126	39,456	33,872	20%
8	2.3	2.48	33,682	29,334	24,892	26%
9	2.1	2.28	43,478	41,226	37,394	14%
10	2.1	2.09	40,126	36,448	33,282	17%
11	2.1	2.12	31,248	27,170	26,415	15%
12	2.1	1.98	38,848	36,465	29,293	25%
13	2.2	2.06	39,146	37,446	31,709	19%
14	2.2	2.24	27,892	25,028	21,342	23%
15	2.7	2.69	44,610	38,364	31,524	29%
16	1.5	1.44	42,222	40,414	31,244	26%
Mean ± SE	2.0±0.3	2.0±0.4	37692±1233	34513±1307	29442±1119	22.2±1.2%

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TABLE 6

**Recovery of Islets Following Freezing and
Thawing in Presence and Absence of Sertoli Cells**

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<u>Islets alone</u>				<u>Islets + Sertoli cells</u>		
No. of islets	Pre-cryo	Post thawing	Recovery (%)	Pre-cryo	Post thawing	Recovery (%)
D3F/D3T	250	152	61%	290	212	73%
	230	131	57%	260	228	88%
	440	278	63%	430	280	88%
10	420	366	87%	410	324	79%
	450	290	64%	440	358	81%
		Means	66.4%			81.8%
D7F/D3T	260	136	52%	250	229	92%
	300	208	69%	300	202	67%
15	280	177	53%	290	238	82%
	360	205	57%	350	300	86%
	320	218	68%	390	289	74%
	380	217	57%	320	270	84%
20		Means	61.0%			80.8%

As shown in Table 5, the yield of islets per gram pancreas was 37692 ± 1233 , 34513 ± 1307 and $29,442 \pm 1119$, after 1, 3 and 7 days of culture, respectively. Following cryopreservation and thawing and reculturing of the cells in the presence of Sertoli cells approximately 20% of the cells were damaged or lost as shown in Table 6. Thus $\pm 24,000$ islets/gram of piglet pancreas were available for transplant purposes after cryopreservation and thawing.

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1 The results showed that insulin secretion
was blunted when glucose was used as insulin
secretagogue prior to cryopreservation. The effect
was more evident following cryopreservation and
5 thawing. While the presence of Sertoli cells had
marked effects on number of islets that survived
cryopreservation and thawing their presence had little
effect on the ability of the islets to respond to a
low glucose concentration as insulin releasing agent.
10 However, as shown in Example 8 the presence of Sertoli
cells augmented the secretion of insulin in the
presence of high glucose concentrations and glucose
plus Forskolin.

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EXAMPLE 5

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**Response of Diabetic Sprague
Dawley Rats to the Transplantation of
Islets from Piglet Donors (Discordant Xenografts)**

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The rats were made diabetic by means of a single i.v. injection of 55 mg/kg of streptozotocin. They were grafted only if the blood sugar was equal to or more than 400 mg/dl. Following transplantation the rats were placed individually in metabolic cages and urine volume, urine glucose content, and body weights were measured at daily intervals. Blood glucose levels were done at weekly intervals. A rat is considered cured of diabetes if the blood glucose level is 160 mg/dl or less and/or the daily urine volume is 15 ml or less.

The results are illustrated in Figures 10 and 11.

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Figure 10 shows the effect of transplantation of piglet islets and Sertoli cells underneath the renal capsule on the mean daily urine output of seven grafted female rat recipients. Each bar represents the mean daily urine output over a ten-day period following transplantation. The study has been conducted over an 80-day period, the bar on the furthest right thus showing the mean urine output per day from day 80 through 89, etc.

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The figure shows that the mean daily urine volume for the first 60 days varied between 19.7 mls and 27 mls or within a diabetic range. It can be readily appreciated that urine volumes decreased to

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1 near-normal levels only from days 70 through day 89.
The corresponding plasma glucose levels during the
first and last ten day periods were 474 ± 46 and $155 \pm$
70, mg/dl, respectively.

5 These results indicate that following
transplantation with piglet islets and Sertoli cells
the rats showed evidence of survival of the grafted
islets. The reversal to normoglycemia took about 80
days.

10 It should be noted that one of the cured
rats is pregnant and has been normoglycemic throughout
her pregnancy.

Figure 11 shows the effect of the
transplantation of piglet islets and Sertoli cells
15 underneath the skin on the mean daily urine volumes of
three rats over a 50 day period. The results show
that the mean urine volume decreased from a mean of
41.7 ml during the first 10-day period to an average
of 12.3 mls during the fifth week. The corresponding
20 glucose levels were 509 ± 45 , and 200 ± 12 , mg/dl,
respectively.

The data depicted above demonstrate that
both the renal subcapsular space and the subcutaneous
area can be used as a site to create an
25 immunologically privileged site for the
transplantation of islet xenografts.

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EXAMPLE 6

5 This study determined the effect of cultured Sertoli cells on the survival of discordant islet xenografts in diabetic rats with minimal early exogenous immunosuppression.

Preparation of Islets

10 Neonatal piglets of less than seven days of age were killed by anesthesia and islets were isolated according to a method of Kuo C.Y., Burghen G.A., Myvacle A. and Herrod H.G. (1994) "Isolation of islets from neonatal pig pancreatic tissue", J. Tissue Culture Methods, 16: 1-7. Briefly, the pancreas was
15 distended by an injection of a collagenase solution, 2 mg/ml, collagenase type X1, in culture medium DMEM. After incubation at 39°C for 17 min, the digested fragments were washed by centrifugation and the digested tissue was then incubated for one week in
20 medium 199 supplemented with 10% horse serum and 1% antibiotics at 32°C. The islets were then cryopreserved according to the method by Lakey J.R.T., Warnock G.L., Kneteman N.M., Ao Z., Rajotte R.V. (1994) "Effects of precryopreservation culture on
25 human islet recovery and in vitro function", Transplant Proc., 26:820 and stored in liquid nitrogen at -196°C. Three days prior to transplantation the cryopreserved islets were rapidly thawed and cultured at 32°C for two days. One day prior to
30 transplantation some of the islets were collected and co-cultured with Sertoli cells for 24 hours.

1 Sertoli cell isolation

Testes of young S-D rats were removed and Sertoli cells were isolated by the method of Cheng C.Y. and Bardin C.W. (1987) "Identification of two
5 testosterone-responsive proteins in Sertoli cell-enriched culture medium whose secretion is suppressed by cells of the intact seminiferous tubule." J. Biol. Chem., 262:12768-12779. Briefly, the testes were digested first in DMEM containing 1.0% trypsin, and
10 then in DMEM containing 1.0% collagenase, type 1, for periods of 15 min each, at 37°C. The purified Sertoli cells were cultured at 37°C in DMEM/F12 supplemented with transferrin, 10 ug/ml, FSH 10 ng/ml, insulin 20 ug/ml and 1.0% FCS, for three days. For
15 transplantation, Sertoli cells and islets were pooled and rats were grafted with either a composite consisting of 5×10^6 Sertoli cells and 3,000 islets, or with islets alone (15 islets/g of body weight).

20 Transplantation of rats

Female S-D rats, weighing between 170 and 200 g were made diabetic by means of a single i.v. injection of 60 mg/kg of streptozotocin. A total of 31 diabetic rats were divided into 3 groups and
25 grafted as follows: Group 1, a control group (n=8), received a total of 15 islets/g body weight injected underneath the renal capsule. No Sertoli cells were grafted. Following transplantation the rats were treated with cyclosporine for 55 days: 25 mg/kg for 3
30 days, 15 mg/kg for 10 days, 10 mg/kg for 10 days and 5 mg/kg for the following 32 days. Immunosuppression

1 was then stopped. Each rat received, in addition, 1-3
U of Ultralente insulin at daily intervals if the 24-
hour urine glucose content exceeded 1 g. Insulin
therapy was stopped on day 55. Group 1, a tissue
5 control group (n=8), was given a renal, subcapsular
injection of a composite of about 5×10^6 Sertoli
cells and 3,000 islets. No CsA was given. Insulin
was given as depicted above. Group 3, the
10 experimental group (n=15), was transplanted with both
sertoli cells and islets and then treated with CsA and
insulin according to the schedule outlined above.

Posttransplantation evaluation of rats

Plasma glucose levels were obtained at
15 weekly intervals. Twenty four hour urine volumes and
urine glucose contents were recorded daily. A rat was
considered cured of the diabetic process if the
following criteria applied: A plasma glucose level of
equal to or less than 10 mmol/L, a 24-hour urine
20 volume of less than 15 ml, and immediate reversal to
hyperglycemia following surgical removal of the
grafted kidney. One normoglycemic rat was mated on day
69 to test her ability to become pregnant.

25 Structural analysis of the grafted tissue

Two normoglycemic rats were nephrectomized
on days 117 and 330 and grafted tissue prepared for
light and electron microscopy. Selawry H.P., Cameron
D.F. (1992) "Sertoli cell-enriched fractions in
30 successful islet cell-transplantation", Cell Trans.,
2:123-129. Briefly, tissue wedges were immersion-

1 fixed with 5% glutaraldehyde in 0.1 M collidine buffer
for 1 h., washed in buffer, and postfixed for 1 h with
1% osmium tetroxide in 0.1 M buffer. Small tissue
blocks were cut from the wedges, and dehydrated
5 through a graded series of ethyl alcohols, transferred
to propylene oxide, and embedded in Epon 812/Araldite
plastic resin. Thick (0.5 um) and thin (900 ng)
sections were stained routinely with toluidine blue
and urinal acetate/lead citrate, respectively, for
10 structural analysis by light and electron microscopy.

The results of the effect of Sertoli cells
and cyclosporine on survival of xenographic
transplantation of pig islet cells into the renal
subcapsular space of diabetic female rats are shown in
15 Table 7.

TABLE 7

Group (n)	Sertoli Cells	CsA	Graft Survival (days)
1 (8)	-	=	0,0,0,0,0,0,0,0
2 (8)	+	-	0,0,0,0,0,0,0,0
3 (15)	+	+	0,0,0,0,0, 71, 77, 96, 117* 148#, >154, >165, >327, 330*

* rats nephrectomized to remove the xenograft

rat died during a cardiac puncture

As shown in Table 7, none of the rats
grafted with islets alone and then given CsA and low-
dose insulin (Group 1) became significantly less
30 hyperglycemic. Further, none of the rats grafted with
a composite of islets and Sertoli cells, but without

1 CsA, showed any improvement of hyperglycemia (Group
2). Of 15 rats grafted with islets and Sertoli cells
and then given CsA (Group 3), 10 showed evidence of
reversal of the diabetic state. Four of the ten are
5 still normoglycemic for periods of more than 154, 165,
165, and 327 days, respectively. The normoglycemic
rats who were nephrectomized on days 117 and 330,
became hyperglycemic immediately. Their plasma
glucose levels were 4.9 mmol/L, and 8.2 mmol/L, prior
10 to, and 20.7 mmol/L, and 32.2 mmol/L, respectively,
following nephrectomy. A female rat who was mated on
day 69 became pregnant and delivered a total of 10
pups on day 89, all of whom she nursed successfully
while remaining normoglycemic. She died on day 148 as
15 a result of a cardiac puncture. Three of 10 rats
regressed into hyperglycemia on days 71, 77, and 96,
respectively, after a short period of euglycemia.

These results demonstrate that prolonged
survival of a discordant islet xenograft (pig to rat)
20 can be achieved in female diabetic rats. Survival of
islet xenografts depended upon two factors which had
to be administered concomitantly: Co-transplantation
with Sertoli cells and treatment with cyclosporine.

The response of total urine volumes
25 following transplantation with a composite of pig
islet and rat Sertoli cells measured at 10-day
intervals over an 80 day period for 7 of the improved
rats showed an average daily urine volume of $27.0 \pm$
13.0 ml/rat during the first 10-day period, which
30 slowly declined to a mean of 12.0 ± 4.0 ml/rat, 70
days following transplantation.

1 Tissue morphology studies shown in Figure 12
show that the tissue and cellular structure of kidney
parenchyma appeared normal in the rat nephrectomized
117 days following transplantation. Normal appearing
5 islets with structurally distinct B-cells were visible
in well vascularized areas subjacent to the kidney
capsule. Additionally, normal appearing Sertoli cells
were observed adjacent to the transplanted islets
along with numerous lymphocytes. No plasma cells were
10 identified at the transplantation site. Viable
endocrine cells were similarly observed in the
subcapsular renal space of the rat nephrectomized 330
days following transplantation.

 These studies show that significant
15 prolongation of survival of a discordant islet
xenograft can be achieved without sustained
immunosuppression. These studies demonstrate that the
mechanism by which Sertoli cells promote islet
xenograft survival is three-fold: (1) Sertoli cells
20 stimulate the recovery of islets damaged during
transplantation (i.e. improve the yield and function
of cultured islets), (2) Sertoli cells protect grafted
islets from immunologic rejection by producing factors
which strongly suppress proliferation of T-cells, and
25 (3) Sertoli cells protect grafted islets from the
toxic effects of cyclosporine.

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EXAMPLE 7

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This study shows a method of isolating and cryopreserving porcine pancreatic islets for future xenographic transplants in mammals.

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Male piglets, < 7 days old and weighing 2± kg were used as donors. The pancreases, weighing 1.4 ± 0.3 g, were harvested and injected with DMEM solution containing 2 mg/ml collagenase XI. The distended pancreas was incubated in a shaking water bath at 39°C for 17 min. The digested tissue was filtered through a 500 µm stainless steel filter and filtrates were washed x 3 with cold DMEM. Without further purification the cells were cultured in M199 and 10% horse serum at 32°C for 7 days. The islet cells were then cryopreserved using standard procedures. At specified intervals islets were thawed and cultured in M199, both in presence, and isolated from testes of male piglets according to a standard method.

25

To test functional capacity, islets cultured for 3 and 7 days were assessed for insulin release in static incubation. In separate experiments, effect of insulin secretagogues was tested on islets cultured with and without Sertoli cells. The results of this study are shown in Tables 8 and 9.

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TABLE 8

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**Effect of Insulin Secretagogues,
Glucose and Glucose Plus Forskolin,
on Insulin Release From Incubated and
Frozen/Thawed (F/T) Islets in the Presence
and Absence of Pig Sertoli Cells**

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	Insulin Release (uU/ml/10islets)		
	3.3mmol/L glucose	16.7 mmol/L glucose	16.7 mmol/L glucose +100 umol Forskolin
Day 3 Incubated with Sertoli cells	42.3 ± 1.2	112.8 ± 17.7*#	267.7 ± 43.0**#
Day 3 Incubated alone	31.3 ± 2.1	57.3 ± 3.8*	123.4 ± 15.3 **
Day 7 Incubated with Sertoli cells	22.9 ± 1.9	64.5 ± 6.4*#	153.9 ± 14.6**
Day 7 Incubated alone	21.3 ± 1.2	37.3 ± 6.0*	120.3 ± 11.4**
Day 3 F/T with Sertoli cells	20.6 ± 4.3	44.9 ± 9.9*	77.1 ± 13.7**
Day 3 F/T alone	11.7 ± 2.3	27.9 ± 6.6*	54.5 ± 10.7**

Anova Test: * vs 3.3mmol/L p 0.5, ** vs both 3.3 & 16.7 mmol/L P<0.05
with Sertoli cells vs islets alone P<0.05

TABLE 9

**Effect of Sertoli cells on insulin content of incubated and
frozen-thawed piglet islets.**

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Insulin content (uU/10 islet(s))		
	Islets alone	Islets & Sertoli cells
Incubated D1	257.0 ± 19.6	391.1 ± 51.4*
Incubated D3	201.1 ± 19.1#	400.1 ± 41.0*#
Incubated D7	179.1 ± 26.2#	271.9 ± 39.9*#
Frozen D3/Thaw D3	52.4 ± 10.3	132.5 ± 35.1
Frozen D7/Thaw D3	10.4 ± 0.9	35.1 ± 8.2

Anova * islets + Sertoli cell vs. islet alone P<0.05

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1 # Incubated islets D3, D7 vs. Frozen D3, D7 P<0.05

 These results show that: (1) large numbers
of neonatal porcine islets can be isolated by a simple
method; (2) cryopreservation and thawing results in
5 about 40% loss in number of islets in the absence of
Sertoli cells and about a 20% loss in the presence of
Sertoli cells ; (3) cultured islets have the ability
to respond to both glucose and glucose + Forskolin as
insulin secretagogues; (4) the functional capacity of
10 the cocultured islet was enhanced two-fold in the
presence of Sertoli cells; (5) following
cryopreservation and thawing, islets recover more
rapidly in presence of Sertoli cells and the response
to both glucose and glucose + Forskolin was enhanced
15 two fold in the presence of Sertoli cells.

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EXAMPLE 8

This example describes a method of treating genetic diabetes which is demonstrated using the animal model NOD (non-Obese Diabetic).

Genetic diabetic mice (NOD) are recipients of pancreatic islet xenografts. Diabetic mice are selected from the colony of NOD mice maintained at the University of Tennessee Medical Center. The current incidence of diabetes in this colony is 80% for females and 63% of males by 25 weeks of age. Animals are considered diabetic if they have two consecutive weekly urine glucose readings of $\geq 3+$ and a confirmatory plasma glucose greater than 400 mg/dl. Three to ten days before transplantation of the graft, the animals receive appropriate insulin to stabilize their health. Diabetic animals are randomly divided into two groups. All animals in these two groups receive 0.3 mg of the antiCD4 antibody, GK1.5 on days -1, 0 and 1 to initiate immunosuppression. Maintenance immunosuppression with GK1.5 cyclosporine A, FK506, cyclophosphamide, rapamycin, nicotinamide or 15-deoxyspergualin may be required.

Porcine pancreatic islets for transplantation are prepared as described in Example 6 except that they are not cryopreserved. Nicotinamide (10mM) or IGF-1 may be added to the incubation medium prior to transplantation. Porcine sertoli cells for transplant are prepared as described in Example 6. Cyclosporine may be included in the culture medium

1 during the first four incubation days prior to
transplantation.

5 One group of the diabetic NOD mice receive
3,000 porcine islets in 25 μ l of Hank's buffered salt
solution (HBSS) under the right renal capsule followed
by an injection of 2×10^7 pig Sertoli cells in 25 μ l
in HBSS under the same renal capsule. A second group
of mice receive a transplant consisting of only the
porcine islet cells. Animals with xenografts continue
10 to receive daily insulin injections; the amount
determined by the concentration of glucose in the
animals urine and plasma. Mice with plasma glucose
levels less than 250 mg/dl are considered cured and no
additional insulin administered.

15 A majority of mice receiving porcine sertoli
cells and porcine pancreatic islets attain normal
urine and plasma glucose levels (xenograft acceptors).
In contrast, the majority of mice receiving porcine
islets alone exhibit graft failure and do not attain
20 normal glucose levels in the urine or plasma.

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EXAMPLE 9

5 This example shows a method of monitoring
the immune response elicited against cellular
transplants.

Total spleen leukocytes are isolated from
mice by methods known in the art from mice that have
rejected their porcine islet xenografts. In
preliminary experiments these leukocytes are
10 administered to NOD/scid mice (NOD mice that in
addition have genetic immunodeficiency) with
successful pig islet xenografts. The leukocytes cause
rejection of the xenograft.

Plastic adherence (to deplete macrophages),
15 nylon wool adherence (to deplete non-T lymphocytes),
or specific antibodies (anti-CD4, anti-CD8, anti-
F4/80, anti-B220) are used to deplete the total
splenocyte preparations of certain classes of
leukocytes. The class-depleted leukocyte preparations
20 are injected into the NOD/scid mice with successful
pig islet xenografts to determine which leukocyte
class is necessary and sufficient to cause xenograft
rejection.

Spleen leukocytes are isolated by
25 conventional methods known in the art from mice
(xenograft acceptor of Example 8) that have accepted
their pig islet xenografts. These leukocytes are
administered to NOD/scid mice with successful pig
islet xenografts. The leukocytes do not cause
30 rejection of the xenograft. Combining the appropriate
leukocyte-depleted preparation that causes rejection

1 with leukocytes from a xenograft acceptor of Example 8
(50/50 mixture) and administering the mixed cell
population to NOD/scid xenograft recipients (adoptive
transfer) allows determination of whether so-called
5 suppressor lymphocytes are preventing xenograft
rejection in xenograft acceptors.

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EXAMPLE 10

5 This example provides a method of encapsulation of Sertoli cells with islets for transplantation.

Preparation of Islets and Sertoli Cell Isolation

10 Islets are isolated from female Fischer rats and are incubated in CMRL medium for approximately four days prior to usage. Sertoli cells are isolated from weanling male Fischer rats and are incubated for approximately four days to confluency in Petri dishes at 37°C.

15 After four days of incubation, the islets are counted and groups of 50 islets are transferred to 24 well Petri dishes. Sertoli cells are removed from Petri dishes with Sigma non-enzymatic media. The Sertoli cells are washed and counted.

20 Three experimental groups are then established as follows: Group 1: 12-well Petri dishes, each well containing 50 islets; Group 2: 12-well Petri dishes, each well containing a total of 1×10^4 Sertoli cells; and Group 3: 12-well Petri dishes, each well containing 50 islets, plus 1×10^4 Sertoli
25 cells. The Petri dishes are incubated at 37°C for 24 hours to permit Sertoli cells to attach to islets.

Microencapsulation of Islets and Sertoli Cells

30 Islets, Sertoli cells and islets plus Sertoli cells are encapsulated by suspension in a solution of sodium alginate which is sprayed into a

1 dish of calcium chloride using a droplet forming
device according to the method of Lim et al. (1980
Science 210:908-910, the contents of which is
incorporated by reference. The droplets are coated
5 with a layer of poly-L-lysine (PLL) with an average
size of 20 kDa at a concentration of 0.05% (w/v) and a
reaction time of 6 minutes according to the method of
Goosen et al. (1985) Biotechnol. Bioeng. 27:146-150,
the contents of which is incorporated by reference.
10 An additional outer layer of sodium alginate is added
around the capsule according to the method of O'Shea
et al. (1984) Biochim. Biophys. Acta 804:133-136,
incorporated herein by reference. Alternatively,
isolated cells may be encapsulated according to the
15 methodology of Weber et al. U.S. Patent No. 5,227,298,
incorporated herein by reference. Following
encapsulation, the cells are divided into treatment
groups. Group 1: Free islets, not encapsulated, Group
2: Islets alone, encapsulated, Group 3: Sertoli cells
20 alone, encapsulated, and Group 4: Islets plus Sertoli
cells encapsulated. Encapsulated cells are placed in
media conventionally selected by the skilled artisan
at 37°C.

25 In Vitro Encapsulation

At specified intervals following
encapsulation, i.e., 1, 7, 14, 21 and 30 days
respectively, following incubation, functionality of
the groups containing islets are examined.
30 Approximately 10 capsules from each of the islet
containing groups are stimulated, in tandem, by a

1 buffered medium containing glucose 9 mmol/L, glucose
at 16.7 mmol/L, and Forskolin at 10mM for 30 minutes,
in a water bath at 37°C. The perfusate is collected
and insulin is assayed using a commercially available
5 kit (e.g., Linco insulin kit). Insulin content of
free encapsulated islets and islets encapsulated with
Sertoli cells is further examined via an acid-ethanol
extract of said capsules and assayed for insulin
content using a commercially available kit (e.g. Linco
10 insulin kit).

In Vivo Encapsulation

Female Wistar-Furth rats are made diabetic
by means of a single i.v. injection of streptozotocin.
15 A total of 32 diabetic rats are divided into four
groups and treated as follows: Group 1, a control
group (n=8), will receive an intraperitoneal injection
of at least 10 capsules containing Sertoli cells
alone; Group 2 (n=8) will receive an intraperitoneal
20 injection of 10 islets/g of free, non-encapsulated
islets; Group 3 (n=8) will receive an i.p. injection
of 10 islets/g of encapsulated islets alone; Group 4,
(n=8) will receive 10 islets/g of co-encapsulated
islets plus Sertoli cells. No group will receive any
25 immunosuppression following transplantation. All rats
are closely monitored via daily plasma glucose levels
for the first week post-transplantation, and then at
weekly intervals thereafter.

Rats are considered cured of diabetes if
30 they exhibit a blood glucose level less than or equal

1 to 170 mg/dl with concomitant steady increases in body weight.

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1 EXAMPLE 11

This example describes a method of immortalizing Sertoli cells using a temperature-sensitive mutant of the SV40 virus.

5 Sertoli cells are isolated from sexually
mature (120 days) rats according to the method of
Wright et al. (1989) Ann. NY Acad. Sci. 564:173-185.
The testes are removed and the tubules are resuspended
in DMEM F-12 (DMEM/F12: Life Technologies, Inc., Grand
10 Island, NY) containing 1mg/ml collagenase
(Worthington, Freehold, NJ), 2mg/ml hyaluronidase
(Sigma, St. Louis, MO), 0.3 mg/ml DNase (Sigma), and
65ug/ml soybean trypsin inhibitor (Sigma), and are
incubated for 25 min at 32°C with gentle shaking. The
15 incubation is repeated and the tubules are washed in
F12/DMEM and digested in an enzyme solution including
1mg/ml collagenase/dipase (Boehringer-Mannheim,
Indianapolis, IN) in lieu of collagenase. The tubules
are recovered and further broken up by gentle
20 pipetting with a Pasteur pipette. The digestion
mixture is filtered through a nylon mesh to remove
clumps of undispersed Sertoli cells. The Sertoli
cells are then sedimented at unit gravity yielding a
95% pure population of adult Sertoli cells.

25 Two 25-cm² flasks are seeded with Sertoli
cells at a density of 5×10^6 cells per plate. These
flasks are incubated with SV40 virus mutant tsA255 for
3 h. The virus-containing medium is removed, and the
cells are incubated at 33°C in F12/DMEM supplemented
30 with 4% fetal bovine serum (FBS). Foci of transformed
cells are visible at 6 weeks after infection. Each

1 individual focus is isolated with sterile metal rings,
the cells are isolated from the plate with trypsin
EDTA, and the cell suspensions are replated in 25-cm²
flasks. Aliquots of cells from each focus are
5 cultured at 33°C or 40°C for two days. At the end of
this culture period, the cells are collected and total
RNA is isolated for Northern blot analysis according
to the methodology of Roberts, et al. (1992) Biol.
Reprod. 47:92-96, incorporated herein by reference.
10 Sertoli cells are selected for cloning on the basis of
the inducible expression of mRNAs encoding Sertoli
cell-secreted proteins according to the methods of
Roberts, et al. (1995) Biol. Reprod. 53:1446-1453,
incorporated herein by reference. Clonal cells are
15 cultured in DMEM/F12 Plus 4% FBS, supplemented with 1%
antibiotic-antimycotic. The cells are then seeded and
allowed to attach at 33°C for at least 24 hours.
Clonal cells are collected by washing the plates twice
with Hanks balanced salt solution followed by a brief
20 incubation with trypsin/EDTA.

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1 WHAT IS CLAIMED IS:

1. A method of treating a disease that results from a deficiency of a biological factor in a mammal wherein said method comprises administering
5 Sertoli cells and a therapeutically effective amount of cells that produce said biological factor to a mammal in need of such treatment, wherein said Sertoli cells are administered in an amount effective to create an immunologically privileged site.
- 10 2. The method of Claim 1 wherein said mammal is a human.
3. The method of Claim 1 wherein said biological factor is a hormone.
4. The method of Claim 1 wherein said
15 biological factor is insulin and said disease is diabetes mellitus.
5. The method of Claim 4 wherein said cells that produce said biological factor are pancreatic islet of Langerhans cells.
- 20 6. The method of Claim 1 wherein said cells that produce said biological factor are cells transformed by a nucleic acid encoding said biological factor.
7. The method of Claim 1 wherein said
25 administering is by transplantation.
8. The method of Claim 1 wherein said Sertoli cells are administered in a dosage ranging from 10^5 to 10^{10} cells.
9. The method of Claim 1 wherein said cells
30 that produce said biological factor are administered in a dosage of from 10^5 to 10^{10} cells.

1 10. The method of Claim 7 wherein said transplantation is by xenograft.

 11. The method of Claim 7 wherein said transplantation is by allograft.

5 12. The method of Claim 1 which further comprises administering an immunosuppressive agent.

 13. The method of Claim 12 wherein said immunosuppressive agent is administered for a time sufficient to permit said transplanted cells to be
10 functional.

 14. The method of Claim 12 wherein said immunosuppressive agent is cyclosporine.

 15. The method of Claim 14 wherein said cyclosporine is administered at a dosage of from 5 to
15 40 mg/kg body wt.

 16. The method of Claim 1 which further comprises administering a therapeutically effective amount of exogenous biological factor following the transplantation of said cells that produce said
20 biological factor.

 17. The method of Claim 1 wherein said cells that produce said biological factor are co-cultured with Sertoli cells in tissue culture.

 18. The method of Claim 17 wherein said
25 cells that produce said biological factor are cryopreserved prior to co-culturing with Sertoli cells in tissue culture.

 19. The method of Claim 1 wherein said Sertoli cells are obtained from a cell line.

30 20. The method of Claim 19 wherein said Sertoli cells are obtained by the steps comprising:

- 1 a. isolating mammalian Sertoli cells from
mammalian tissue;
- b. incubating said isolated mammalian
Sertoli cells with virus producing cells under
5 conditions sufficient to transform said Sertoli cells;
- c. isolating said transformed Sertoli cells
from the virus producing cell; and
- d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
10 cloning.
21. The method of Claim 19 wherein said
Sertoli cells are obtained by the steps comprising:
- a. isolating mammalian Sertoli cells from
mammalian tissue;
- 15 b. incubating said isolated mammalian
Sertoli cells with a mutagen under conditions
sufficient to transform said Sertoli cells;
- c. collecting said transformed Sertoli
cells; and
- 20 d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
cloning.
22. The method of Claim 20, wherein said
virus producing cells are SV40 or polyoma virus.
- 25 23. The method of Claim 1, wherein the
Sertoli cells and the cells that produce a biological
factor are co-localized.
24. The method of Claim 23, wherein the
Sertoli cells and the cells that produce a biological
30 factor are co-encapsulated.

1 25. A method of treating diabetes mellitus
in a mammal wherein said method comprises
administering to a diabetic mammal Sertoli cells in an
amount effective to create an immunologically
5 privileged site and a therapeutically effective amount
of pancreatic islet of Langerhans cells.

26. The method of Claim 25 wherein said
diabetes mellitus is type I or type II.

10 27. The method of Claim 25 wherein said
mammal is a human.

28. The method of Claim 25 wherein said
Sertoli cells are human, bovine or porcine.

15 29. The method of Claim 25 wherein said
pancreatic islet of Langerhans cells are human, bovine
or porcine.

30. The method of Claim 25 wherein said
administering is by transplantation.

20 31. The method of Claim 30 wherein said
transplantation is by injection into the renal
subcapsular space.

32. The method of Claim 30 wherein said
transplantation is by injection into the subcutaneous
facie.

25 33. The method of Claim 25 wherein said
Sertoli cells are administered at a dosage ranging
from 10^5 to 10^{10} cells.

30 34. The method of Claim 25 wherein said
islet of Langerhans cells are administered at a dosage
ranging from 5-1000 islet cells/g body wt.

1 35. The method of Claim 25 which further
comprises the administration of an immunosuppressive
agent.

5 36. The method of Claim 35 wherein said
immunosuppressive agent is administered for a time
sufficient to permit the transplanted islets to be
functional.

37. The method of Claim 35 wherein said
immunosuppressive agent is cyclosporine.

10 38. The method of Claim 35 wherein said
cyclosporine is administered at a dosage of 5 to 40
mg/kg body wt.

15 39. The method of Claim 25 which further
comprises administering a therapeutically effective
amount of insulin following transplantation of said
pancreatic islet of Langerhans cells.

40. The method of Claim 25 wherein said
Sertoli cells are obtained from a cell line.

20 41. The method of Claim 40 wherein said
Sertoli cells are obtained by the steps comprising:

 a. isolating mammalian Sertoli cells from
mammalian tissue;

25 b. incubating said isolated mammalian
Sertoli cells with virus producing cells under
conditions sufficient to transform said Sertoli cells;

 c. isolating said transformed Sertoli cells
from the virus producing cell; and

30 d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
cloning.

- 1 42. The method of Claim 40 wherein said
Sertoli cells are obtained by the steps comprising:
- 5 a. isolating mammalian Sertoli cells from
mammalian tissue;
- b. incubating said isolated mammalian
Sertoli cells with a mutagen under conditions
sufficient to transform said Sertoli cells;
- c. collecting said transformed Sertoli
cells; and
- 10 d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
cloning.

 43. The method of Claim 41 wherein said
virus producing cells are SV40 or polyoma virus.

15 44. The method of Claim 25, wherein the
Sertoli cells and the pancreatic islet of Langerhans
cells are co-localized.

 45. The method of Claim 25, wherein the
Sertoli cells and the pancreatic islet of Langerhans
20 cells are co-encapsulated.

 46. A method of creating an immunologically
privileged site in a mammal wherein said method
comprises transplanting isolated Sertoli cells into a
mammal.

25 47. The method of Claim 46 wherein said
mammal is a human.

 48. The method of Claim 46 wherein said
Sertoli cells are injected into the renal subcapsular
space.

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1 49. The method of Claim 46 wherein said
Sertoli cells are injected into the subcutaneous
facie.

5 50. The method of Claim 46 wherein said
Sertoli cells are transplanted at a dosage ranging
from 10^5 to 10^{10} cells.

51. The method of Claim 46 wherein said
Sertoli-cells are human, bovine or porcine.

10 52. The method of Claim 46 wherein said
Sertoli cells are obtained from a cell line.

53. The method of Claim 50 wherein said
Sertoli cells are obtained by the steps comprising:

- a. isolating mammalian Sertoli cells from
mammalian tissue;
- 15 b. incubating said isolated mammalian
Sertoli cells with virus producing cells under
conditions sufficient to transform said Sertoli cells;
- c. isolating said transformed Sertoli cells
from the virus producing cell; and
- 20 d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
cloning.

54. The method of Claim 50 wherein said
Sertoli cells are obtained by the steps comprising:

- 25 a. isolating mammalian Sertoli cells from
mammalian tissue;
- b. incubating said isolated mammalian
Sertoli cells with a mutagen under conditions
sufficient to transform said Sertoli cells;
- 30 c. collecting said transformed Sertoli
cells; and

1 d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
cloning.

5 55. The method of Claim 53 wherein said
virus producing cells are SV40 or polyoma virus.

10 56. A method of creating systemic tolerance
to subsequent transplants comprising transplanting
Sertoli cells prior to said subsequent transplant with
a transplant in an amount sufficient to tolerize said
mammal.

15 57. The method of Claim 56 wherein said
transplants are endocrine cells.

20 58. The method of Claim 56 wherein said
Sertoli cells are administered in a dosage ranging
from 10^5 to 10^{10} cells.

25 59. The method of Claim 56 wherein
endocrine cells are simultaneously transplanted with
said Sertoli cells.

30 60. The method of Claim 56 wherein said
Sertoli cells are obtained from a cell line.

35 61. The method of Claim 60 wherein said
Sertoli cells are obtained by the steps comprising:

 a. isolating mammalian Sertoli cells from
mammalian tissue;

40 b. incubating said isolated mammalian
Sertoli cells with virus producing cells under
conditions sufficient to transform said Sertoli cells;

 c. isolating said transformed Sertoli cells
from the virus producing cell; and

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1 d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
cloning.

62. The method of Claim 50 wherein said
5 Sertoli cells are obtained by the steps comprising:

- a. isolating mammalian Sertoli cells from
mammalian tissue;
- b. incubating said isolated mammalian
Sertoli cells with a mutagen under conditions
10 sufficient to transform said Sertoli cells;
- c. collecting said transformed Sertoli
cells; and
- d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
15 cloning.

63. The method of Claim 61 wherein said
virus producing cells are SV40 or polyoma virus.

64. A method of treating an autoimmune
disease in a mammal wherein said method comprises
20 administering to said mammal a therapeutically
effective amount of Sertoli cells.

65. The method of Claim 64 wherein said
Sertoli cells are administered in a dosage ranging
from 10^5 to 10^{10} cells.

25 66. The method of Claim 64 wherein said
Sertoli cells are obtained from a cell line.

67. The method of Claim 64 wherein said
Sertoli cells are obtained by the steps comprising:
a. isolating mammalian Sertoli cells from
30 mammalian tissue;

- 1 b. incubating said isolated mammalian
Sertoli cells with virus producing cells under
conditions sufficient to transform said Sertoli cells;
 c. collecting said transformed Sertoli
5 cells from the virus producing cell; and
 d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
cloning.

68. The method of Claim 64 wherein said
10 Sertoli cells are obtained by the steps comprising:
 a. isolating mammalian Sertoli cells from
mammalian tissue;
 b. incubating said isolated mammalian
Sertoli cells with a mutagen under conditions
15 sufficient to transform said Sertoli cells;
 c. isolating said transformed Sertoli
cells; and
 d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
20 cloning.

69. The method of Claim 67 wherein said
virus producing cells are SV40 or polyoma virus.

70. A method of enhancing the maturation,
proliferation and functional capacity of mammalian
25 cells in tissue culture comprising co-culturing said
cells with Sertoli cells.

71. The method of Claim 70 wherein said
Sertoli cells are co-cultured in an amount ranging
from 10^5 to 10^{10} cells.

- 30 72. The method of Claim 70 wherein said
Sertoli cells are obtained from a cell line.

- 1 73. The method of Claim 72 wherein said
Sertoli cells are obtained by the steps comprising:
- a. isolating mammalian Sertoli cells from
mammalian tissue;
 - 5 b. incubating said isolated mammalian
Sertoli cells with virus producing cells under
conditions sufficient to transform said Sertoli cells;
 - c. isolating said transformed Sertoli cells
from the virus producing cell; and
 - 10 d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
cloning.

74. The method of Claim 72 wherein said
Sertoli cells are obtained by the steps comprising:
- 15 a. isolating mammalian Sertoli cells from
mammalian tissue;
 - b. incubating said isolated mammalian
Sertoli cells with a mutagen under conditions
sufficient to transform said Sertoli cells;
 - 20 c. collecting said transformed Sertoli
cells; and
 - d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
cloning.

- 25 75. The method of Claim 70 wherein said
virus producing cells are SV40 or polyoma virus.

76. The method of Claim 70 wherein the
mammalian cells and the Sertoli cells are co-
localized.

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1 77. The method of Claim 70 wherein the
mammalian cells and the Sertoli cells are co-encapsulated.

5 78. A method of enhancing the recovery rate
and viability of frozen mammalian cells in tissue
culture-comprising co-culturing said cells with
Sertoli cells.

 79. The method of Claim 78 wherein said
mammalian cells are endocrine cells or germ cells.

10 80. A method of enhancing the recovery and
proliferation of ex vivo cells comprising co-culturing
said cells with Sertoli cells for a time and under
conditions sufficient to achieve said enhanced
recovery and proliferation.

15 81. A method of enhancing the recovery and
proliferation of ex vivo cells comprising culturing
said cells with a culture media from a tissue culture
containing Sertoli cells for a time and under
conditions sufficient to achieve said enhanced
recovery and proliferation.

20 82. A pharmaceutical composition comprising
Sertoli cells and cells that produce a biological
factor and a pharmaceutically acceptable carrier.

 83. The composition of Claim 82 wherein
said biological factor is a hormone.

25 84. The composition of Claim 82 wherein
said cells that produce a biological factor are
pancreatic islet of Langerhans cells.

30 85. The composition of Claim 82 wherein
said cells that produce said biological factor are
cells that are transformed by a nucleic acid encoding
said biological factor.

1 86. The composition of Claim 82 wherein
said Sertoli cells are obtained from a cell line.

 87. The composition of Claim 82 wherein
said Sertoli cells are obtained by the steps
5 comprising:

- a. isolating mammalian Sertoli cells from
mammalian tissue;
- b. incubating said isolated mammalian
Sertoli cells with virus producing cells under
10 conditions sufficient to transform said Sertoli cells;
- c. isolating said transformed Sertoli cells
from the virus producing cell; and
- d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
15 cloning.

 88. The composition of Claim 82 wherein
said Sertoli cells are obtained by the steps
comprising:

- a. isolating mammalian Sertoli cells from
20 mammalian tissue;
- b. incubating said isolated mammalian
Sertoli cells with a mutagen under conditions
sufficient to transform said Sertoli cells;
- c. collecting said transformed Sertoli
25 cells; and
- d. optionally screening transformed Sertoli
cells for expression of an appropriate isolate for
cloning.

 89. The composition of Claim 82 wherein
30 said virus producing cells are SV40 or polyoma virus.

 90. A pharmaceutical composition comprising

1 Sertoli cells, pancreatic islet of Langerhans cells
and a pharmaceutically acceptable carrier.

5 91. A pharmaceutical composition comprising
Sertoli-cells and a pharmaceutically acceptable
carrier.

92. A compartmentalized kit adapted to
receive a first container adapted to contain Sertoli
cells and a second container adapted to contain cells
that produce a biological factor that is absent or
10 defective in a disease.

93. A compartmentalized kit adapted to
receive a first container adapted to contain Sertoli
cells and a second container adapted to contain
pancreatic islet of Langerhans cells.

15 94. An article of manufacture comprising a
packaging material and Sertoli cells contained within
said packaging material, wherein said Sertoli cells
are effective for creating an immunologically
privileged site in a mammal, and wherein said
20 packaging material contains a label that indicates
that said Sertoli cells can be used for creating an
immunologically privileged site in a mammal.

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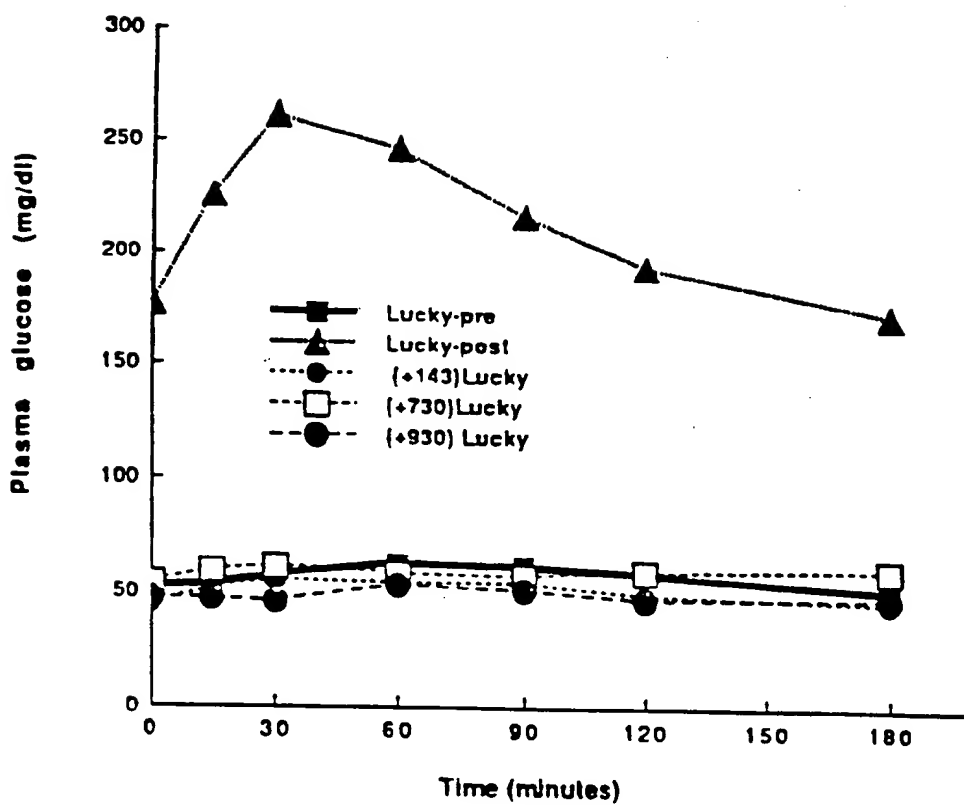


FIGURE 1

2 / 1 2

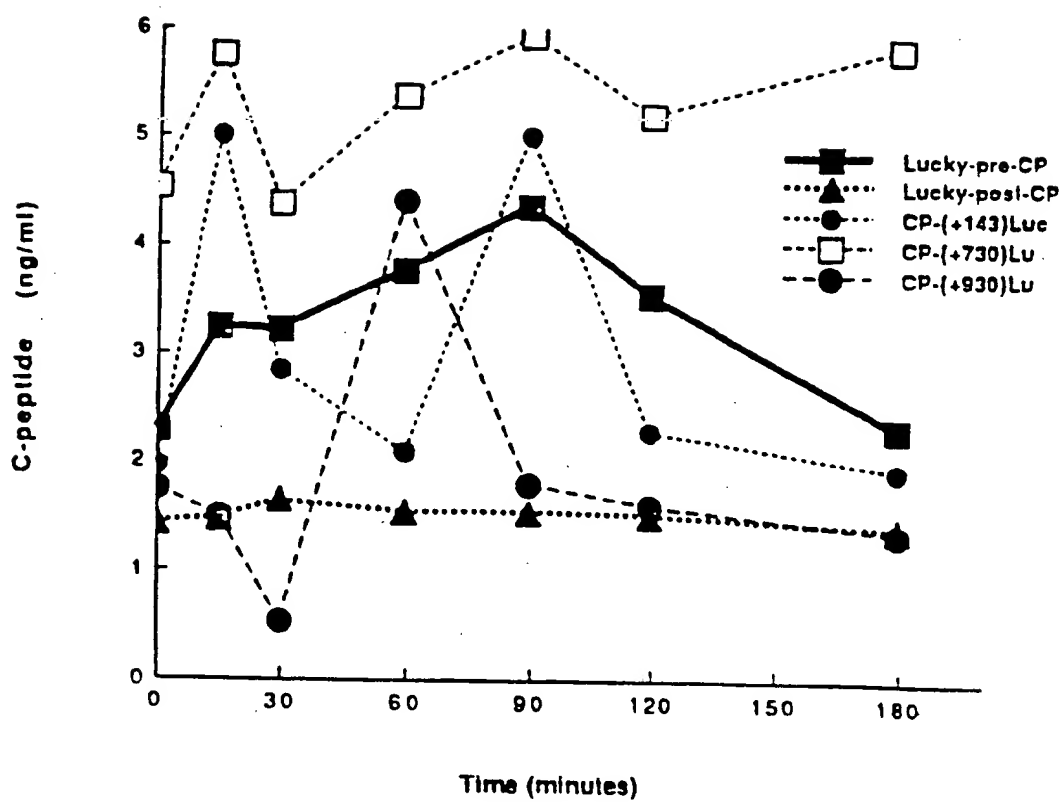


FIGURE 2

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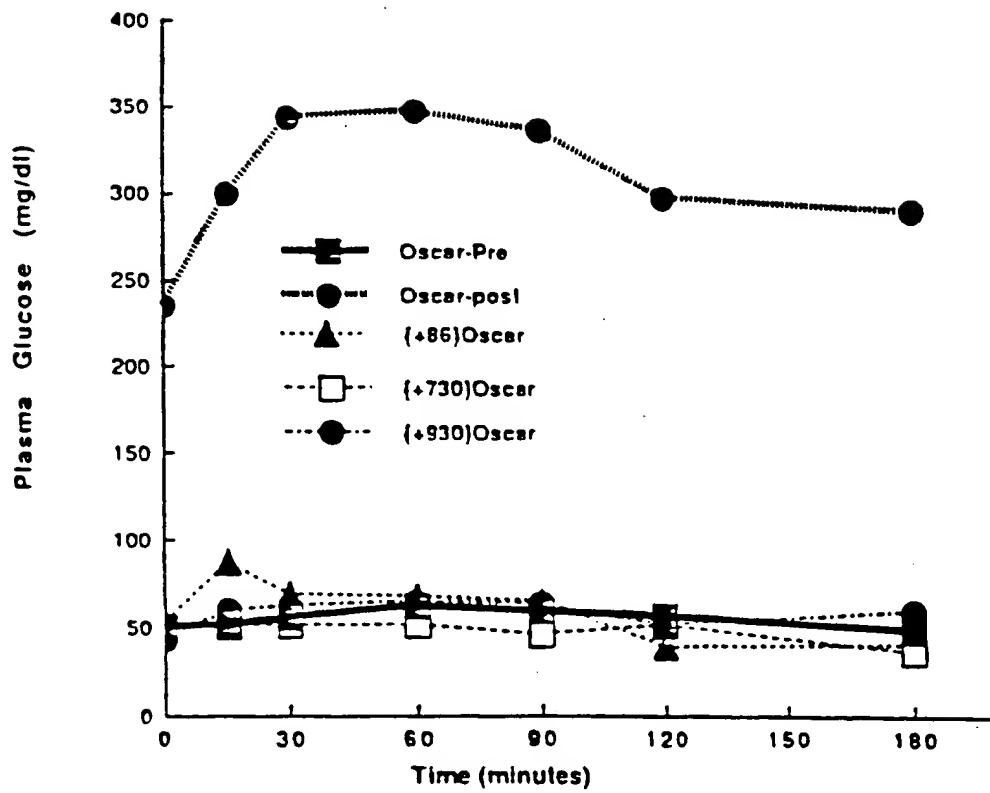


FIGURE 3

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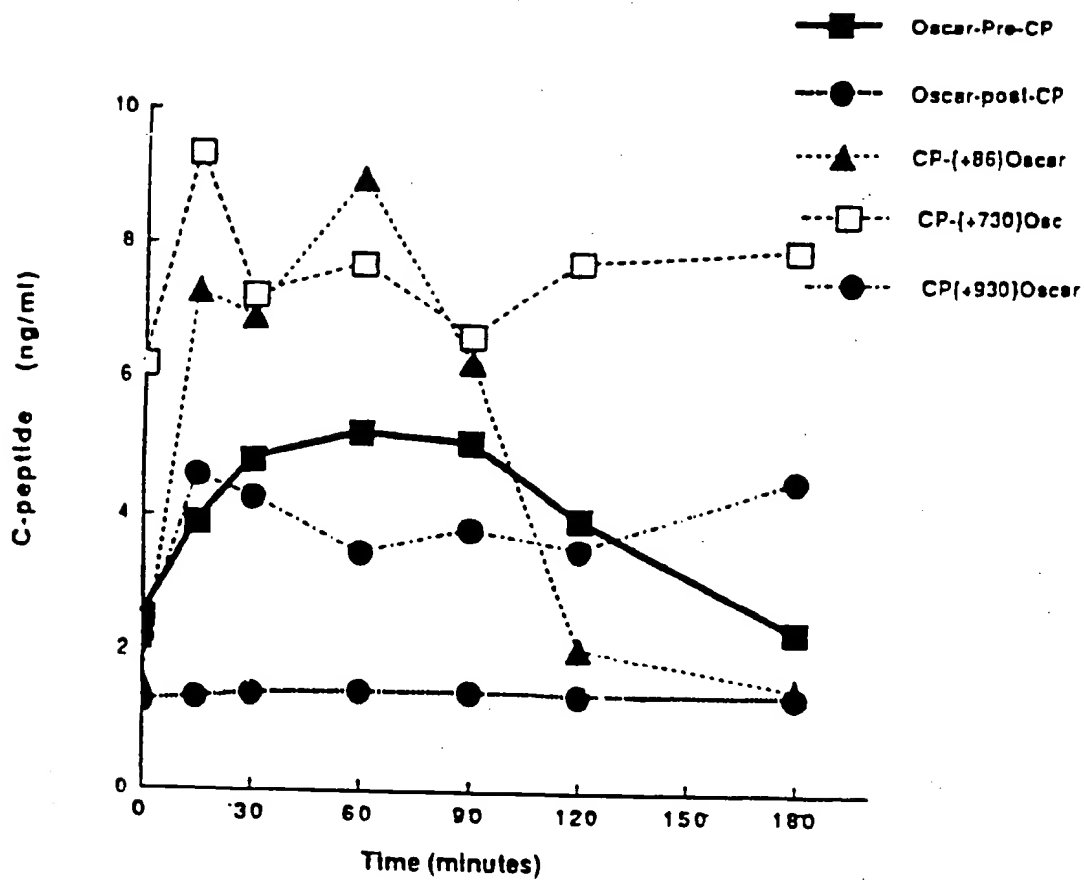
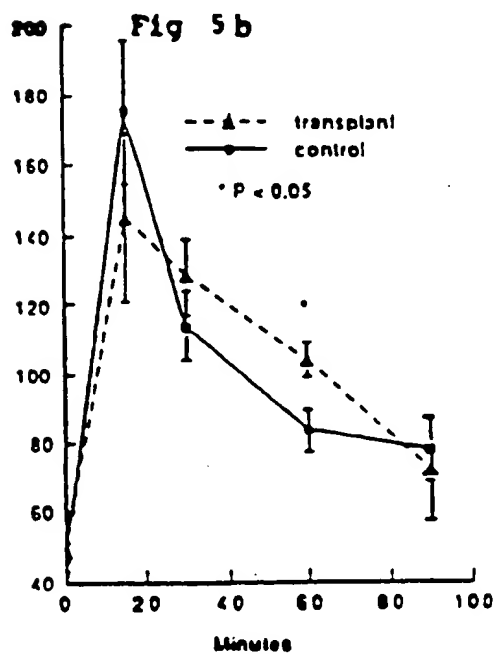
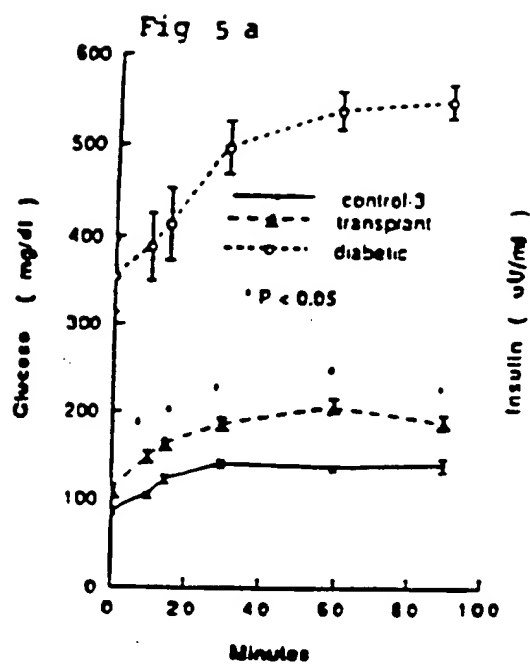


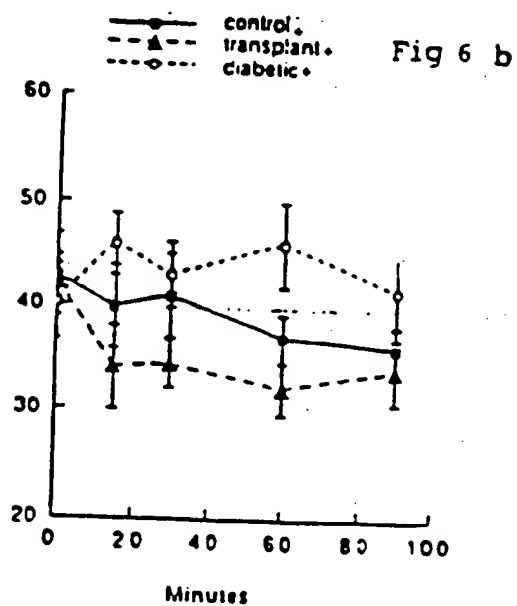
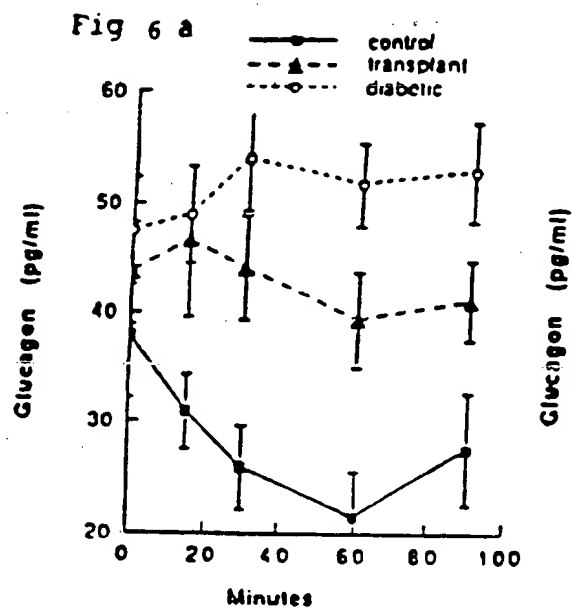
FIGURE 4

5 / 12



FIGURES 5a and 5b

6 / 1 2



FIGURES 6a and 6b

7/12



FIG. 7

8/12

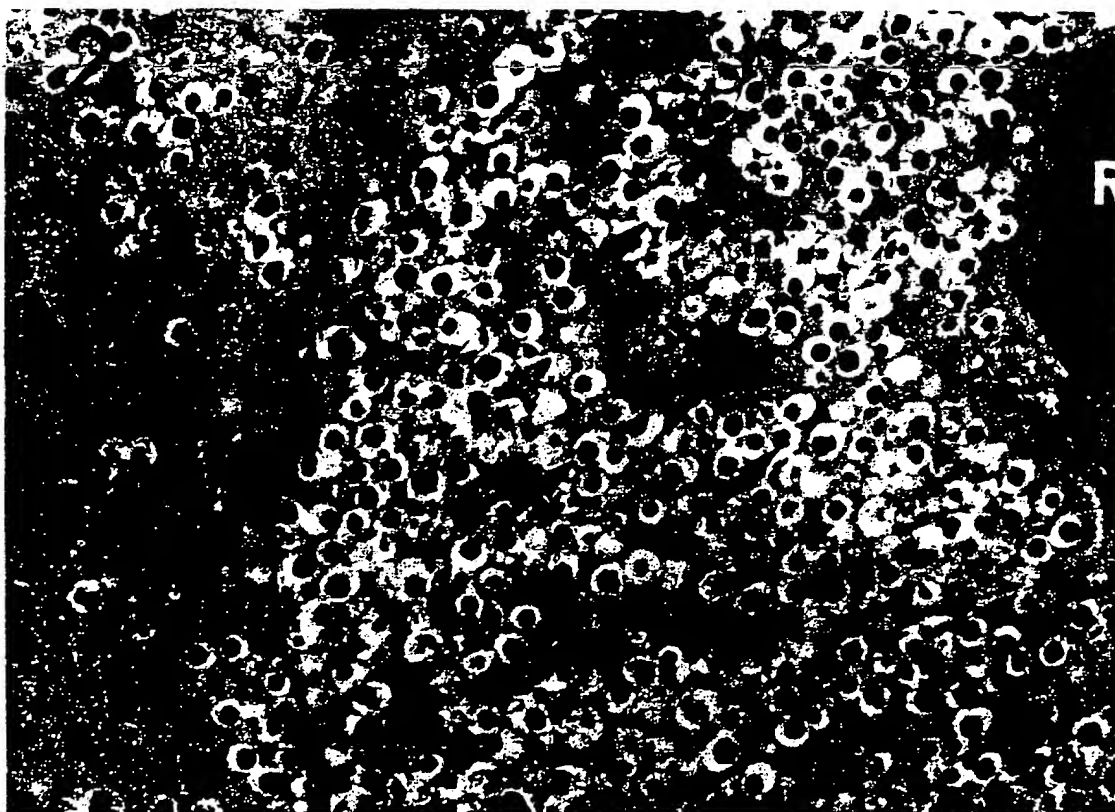


FIG. 8

9/12

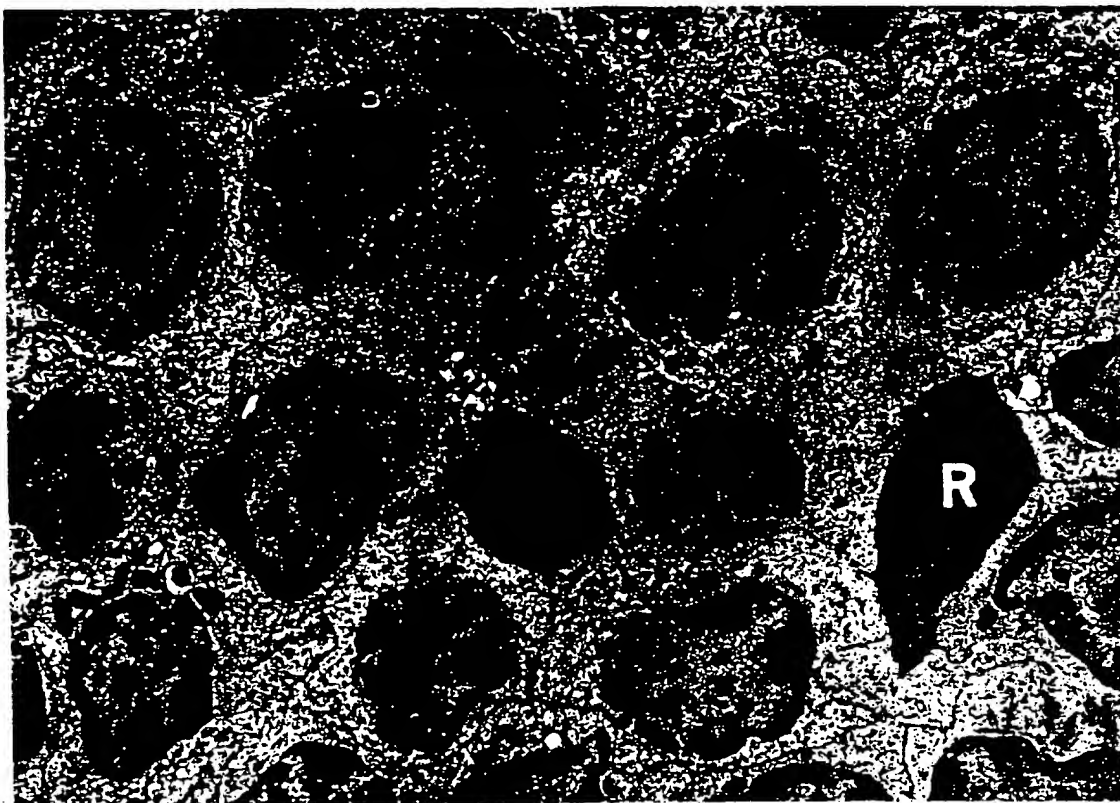


FIG. 9

10 / 12

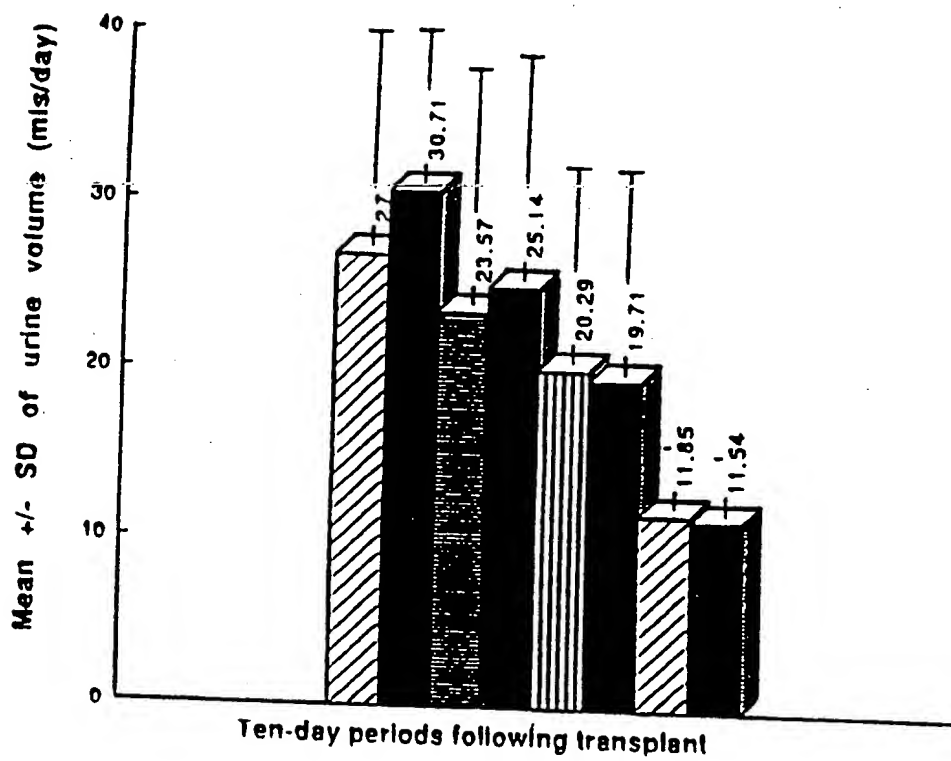


FIGURE 10

11/12

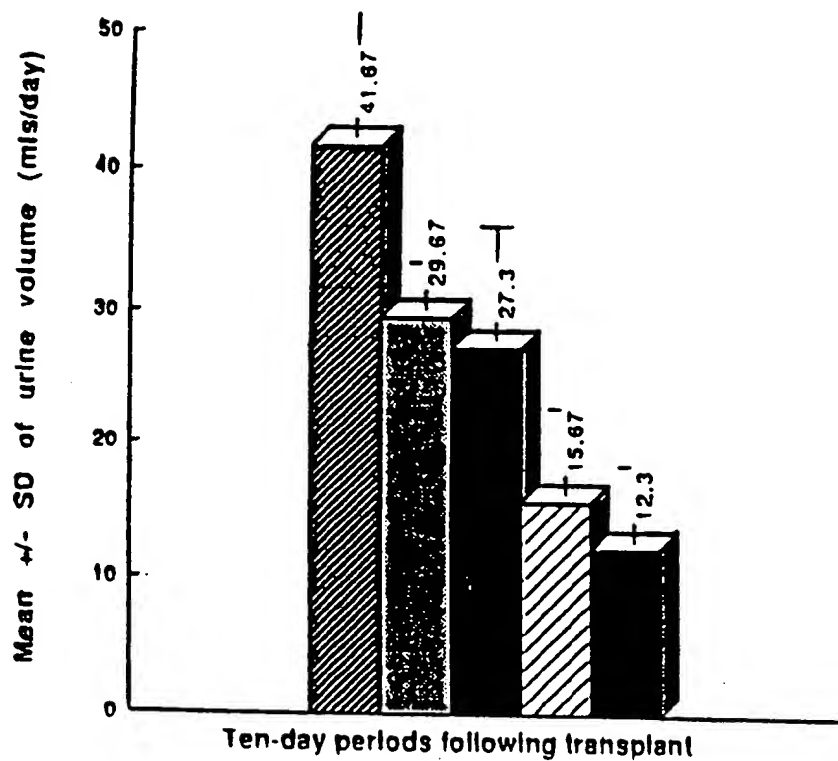


FIGURE 11

12/12

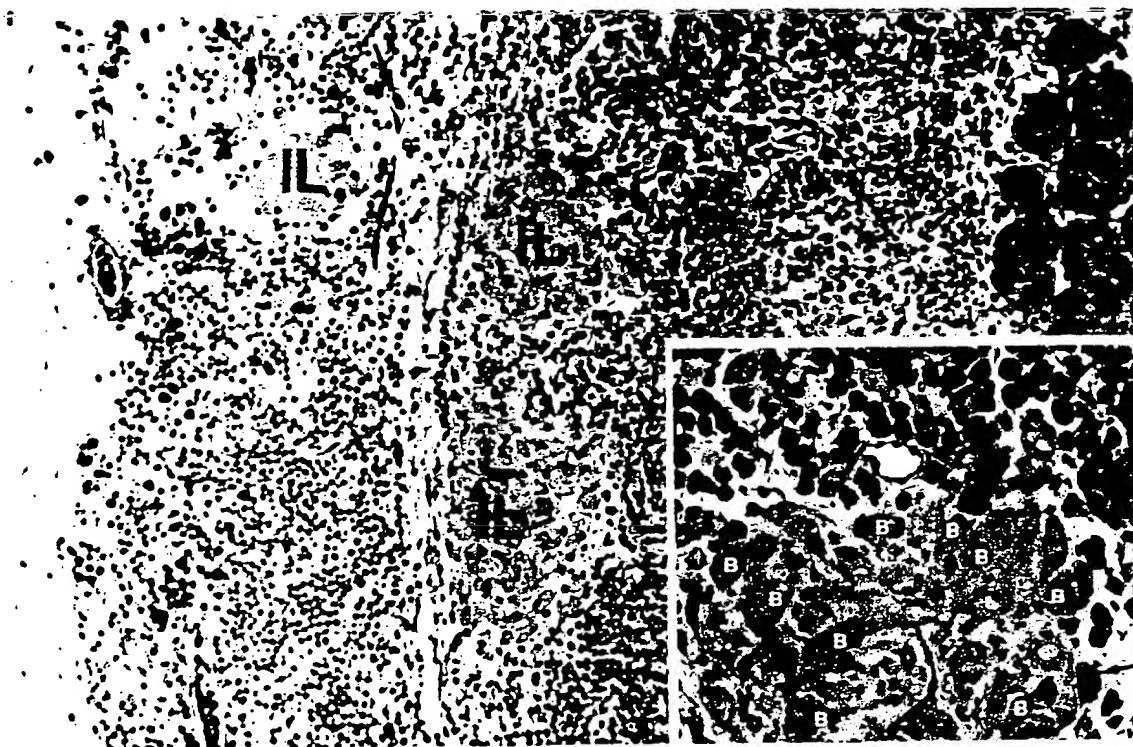


FIG. 12

INTERNATIONAL SEARCH REPORT

International Application No

PC, /US 96/09627

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K35/48 C12N5/08 //(A61K35/48,A61K35:39)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>CELL TRANSPLANTATION, vol. 2, no. 2, March 1993, pages 123-129, XP000602285 SELAURY ET AL: " SERTOLI CELL-ENRICHED FRACTIONS IN SUCCESSFUL ISLET CELL TRANSPLANTATION" cited in the application see the whole document</p> <p>---</p> <p>-/--</p>	1-94



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

Date of the actual completion of the international search

7 October 1996

Date of mailing of the international search report

18. 10. 96

Name and mailing address of the ISA

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Fax (+ 31-70) 340-3016

Authorized officer

Sitch, W

INTERNATIONAL SEARCH REPORT

International Application No
PC1/US 96/09627

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>TRANSPLANTATION, vol. 52, no. 5, November 1991, pages 846-850, XP000602288 SELAURY ET AL: " PRODUCTION OF A FACTOR,OR FACTORS,SUPPRESSING IL-2 PRODUCTION AND T CELL PROLIFERATION BY SERTOLI CELL-ENRICHED PREPARATIONS.A POTENTIAL ROLE FOR ISLET TRANSPLANTATION IN AN IMMUNOLOGICALLY PRIVILEGED SITE" see the whole document</p> <p>---</p>	1-94
X	<p>JOURNAL OF UROLOGY, vol. 134, 1985, pages 782-786, XP000602284 WHITMORE III ET AL: "THE ROLE OF GERMINAL EPITHELIUM AND SPERMATOGENESIS IN THE PREVILEGED SURVIVAL OF INTRATESTICULAR GRAFTS" see the whole document</p> <p>---</p>	1-94
X	<p>DATABASE MEDLINE FILE SERVER STN KARLSRUHE ABSTRACT 92347475, HOFMANN ET AL: "IMMORTALIZATION OF GERM CELLS AND SOMATIC TESTICULAR CELLS USING THE SV40 LARGE T ANTIGEN" XP002015232</p> <p>---</p>	70-81
A	<p>& EXPERIMENTAL CELL RESEARCH, (1992 AUG) 201(2) 417-35 see abstract</p> <p>---</p>	20,22, 41,43, 53,55, 61,63, 67,69, 73,75, 87,89
X	<p>DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US ABSTRACT 93:280614, LEJEUNE ET AL: "ENHANCEMENT OF TESTOSTERONE SECRETION BY NORMAL ADULT HUMAN LEYDIG CELLS BY CO-CULTURE WITH ENRICHED PREPARATIONS OF NORMAL ADULT HUMAN SERTOLI CELLS" XP002015233 & INT J ANDROL 16(1).1993.27-34 see abstract</p> <p>---</p>	70-81

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INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 96/09627

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US ABSTRACT 92:167853, BERENDS ET AL: "SIGNIFICANT IMPROVEMENT OF THE SURVIVAL OF SEMINOMA CELLS IN-VITRO BY USE OF A RAT SERTOLI CELL FEEDER LAYER AND SERUM-FREE MEDIUM" XP002015234 & J NATL CANCER INST (BETHESDA) 83(19).1991. 1400-1403. see abstract</p> <p>---</p>	70-81
X	<p>DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US ABSTRACT 88:227147, CARREAU ET AL: "STIMULATION OF ADULT RAT LEYDIG CELL AROMATASE ACTIVITY BY A SERTOLI CELL FACTOR" XP002015235 & ENDOCRINOLOGY 122(3).1988.1103-1109 see abstract</p> <p>---</p>	70-81
A	<p>MOLECULAR AND CELLULAR ENDOCRINOLOGY, vol. 55, 1988, pages 33-43, XP000602963 OONK ET AL: "INSULIN-LIKE GROWTH FACTOR I (IGF-I) RECEPTORS ON SERTOLI CELLS FROM IMMATURE RATS AND AGE-DEPENDENT TESTICULAR BINDING OF IGF-I AND INSULIN"</p> <p>---</p>	
P,X	<p>WO,A,95 28617 (BALCO INC ;KREFT KEITH A (US); HEINRICH KARL F (US)) 26 October 1995 see the whole document</p> <p>---</p>	1-94
P,A	<p>BIOLOGY OF REPRODUCTION, vol. 53, December 1995, pages 1446-1453, XP000602206 ROBERTS ET AL: "IMMORTALIZATION AND CHARACTERIZATION OF A SERTOLI CELL LINE FROM THE ADULT RAT" cited in the application see the whole document</p> <p>-----</p>	20,22, 41,43, 53,55, 61,63, 67,69, 73,75, 87,89

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/ 09627

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-69
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Information on patent family members

PCT/US 96/09627

Form PCT/ISA/210 (patent family annex) (July 1992)